Medical Lib

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CONTENTS

	PAGE
Reactions to Hemorrhage. *Walter J. Meek and J. A. E. Eyster*	1
Further Experiments on the Activation of Muscle Catalase by Liver. *Theo. C. Burnett*	16
The Source of Diastases of the Blood. *L. H. Davis and Ellison L. Ross*	22
Changes in the Catalase Content During the Life Cycle. *W. E. Burge and E. L. Burge*	29
The Nutritive Value of Yeast in Bread. *Philip B. Hawk, Clarence A. Smith and Olaf Bergeim*	33
The Effect of Suckling and Castration on the Lactating Mammary Gland in Rat and Guinea Pig. *Choizu Kuramitsu and Leo Loeb*	40
Diet and Sex as Factors in the Creatinuria of Man. *Genevieve Stearns and Howard B. Lewis*	60
Studies on the Visceral Sensory Nervous System. VIII. On the Presence of Vasomotor Fibers in the Vagus Nerve to the Pulmonary Vessels of the Amphibian and the Reptilian Lung. *A. B. Luckhardt and A. J. Carlson*	72
Paradoxical Pupil Dilatation Following Lesions of the Afferent Paths. *Joseph Byrne*	113
Fatigue in Frog Muscle when Immersed in Various Concentrations of Lipoid-Solvents; Especially the Higher Alcohols. *Francis Marsh Baldwin*	127
Amoeboid Movement, Tissue Formation and Consistency of Protoplasm. *Leo Loeb*	140
A Study of the Distribution of Iodine Between Cells and Colloid in the Thyroid Gland. III. The Effect of Stimulation of the Vago-Sympathetic Nerve on the Distribution and Concentration of Iodine in the Dog's Thyroid Gland. *Harry Benjamin van Dyke*	168
Extensibility of Muscle: The Effect of Stretching upon the Development of Fatigue in a Muscle. *Nathan B. Eddy and Ardrey W. Downs*	182
Extensibility of Muscle: The Production of Carbon Dioxide by a Muscle when it is Made to Support a Weight. *Nathan B. Eddy and Ardrey W. Downs*	188
Studies of the Thyroid Apparatus. I. The Stability of the Nervous System as a Factor in the Resistance of the Albino Rat to the Loss of the Parathyroid Secretion. *Frederick S. Hammett*	196
Studies in Nutrition. VI. The Nutritive Value of the Proteins of the Lima Bean, Phaseolus Lunatus. *A. J. Finks and Carl O. Johns*	205
Studies in Nutrition. VII. The Nutritive Value of the Proteins of the Adsuki Bean, Phaseolus Angularis. *Carl O. Johns and A. J. Finks*	208
The Epinephrin Output Estimated by Collecting the Adrenal Blood without Opening the Abdomen. *G. N. Stewart and J. M. Rogoff*	213
Post-Operative Depletion of the Epinephrin Store of the Adrenals. *G. N. Stewart and J. M. Rogoff*	220

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PURPOSES

The main purpose of the Physiological Reviews is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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J. A. E. Eyster and W. J. Meek: The Origin and Propagation of the Cardiac Impulse

H. Gideon Wells: The Anaphylactic Reaction

Charles Sheard: Photo-Electric Currents in the Eye

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APRIL

Joseph Erlanger: Blood Volume and its Regulation

J. J. R. Macleod: The Sugar of the Blood

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Henry G. Barbour: The Heat Regulating Mechanism of the Body

Gilbert Horrax: Contributions of War Surgery to the Physiology of the (Central) Nervous System

JULY

P. A. Levene: Structure and Significance of the Phosphatids

H. D. Dakin: Physiological Oxidations

E. G. Martin: Tests for Muscular Efficiency

Samuel Goldschmidt: Intestinal Absorption

A. J. Carlson: Gastric Secretion in Health and Disease

OCTOBER

S. Walter Ranson: The Afferent Paths for the Visceral Reflexes

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REACTIONS TO HEMORRHAGE

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From the Physiological Laboratory of the University of Wisconsin Medical School

Received for publication December 17, 1920

Many of the physiological reactions to hemorrhage have long been known and carefully studied. It was early recognized that after any considerable loss of blood, body fluids enter the circulation in an attempt to restore volume. With the development of blood pressure reading devices, it was found that the vasomotor center tends to bring about a general constriction of the peripheral blood vessels, which compensates for the decreased viscosity of the blood and diminishes the capacity of the circulatory system so that blood pressure may be maintained and an adequate supply of blood furnished to the central nervous system and other vital organs. The larger arteries also passively accommodate themselves to the decreased volume. Arterial blood pressure remains practically constant until blood equalling 2 to 3 per cent of the body weight is lost. The pulse rate increases with the advancing hemorrhage due most likely to decreased vagal tonus. A faster respiratory rate may facilitate venous return. Although it is obvious that sooner or later the curtailment of venous return and the interference with diastolic filling of the ventricle must decrease the cardiac output, actual experiments on the part played by the heart in the compensations to hemorrhage have been very few and the exact point at which hemorrhage decreases output has never been determined. Johansson and Tigerstedt (1), using the pericardial sac as an uncalibrated cardiometer, showed that the heart empties itself more completely after hemorrhage. Wiggers (2), (3) in excellent reviews of the literature and studies of his own on hemorrhage, deduced from pulse pressure tracings that a latent period of a few seconds to several minutes might intervene before the filling of the ventricles was affected, this being due to the luxus

supply of blood in the venous cisterns temporarily supplying the deficit. Although Wiggers (2) states that he compared pulse pressure tracings with volume outputs as determined by a cardiometer, the data were not presented and so far as we can find there are no papers in the literature of hemorrhage in which systolic output or heart size have been directly studied.

The work presented here is briefly the effect of successive small hemorrhages on the diastolic size of the heart as determined by the x-ray, and a further investigation of the problem by the more usual laboratory methods such as the cardiometer and arterial and venous manometers.

Methods. Dogs were used in all experiments. For the x-ray examinations they were morphinized and tied to an operating board. By means of a plumb bob and a marked spot on the dog between the shoulder blades the animal could be placed under the tube in exactly the previous position. This point was always further tested by seeing if the successive negatives would superimpose, using the shadows of the ribs as guide marks. In this way one could be sure that any variation in the outline of the heart was due to a change in the heart itself and not to distortion resulting from a different position of the animal. These precautions, which keep the heart in its natural place in the thorax and rigidly control the series of x-ray photographs, are believed to be of great importance in experimental work of this kind. The pictures were made during successive inspirations with two or three short flashes from a Coolidge tube, the spark gap being about 4½ inches and the current about 35 milliamperes.

The cannula for bleeding was placed in the femoral artery, the incision being made under cocaine. Bleeding was rapid, seldom lasting over 1 minute. X-ray plates were made before, during and at intervals after the hemorrhage.

X-rays taken as described give the diastolic shadow of the heart. For comparison the heart shadows were measured with a planimeter. A variation in the silhouette area is of course taken to mean a corresponding change in heart volume.. With good films the areas can be marked off with very little error. In the dog's heart, where the apex often lies well above the diaphragm in inspiration, the only part of the outline left to the experimenter's judgment is the line across the base. If this be carried across with reference to the same intercostal space the error from this source in successive films is negligible. It is possible to take a series of observations on a normal dog with variations of only about 5 per cent.

In the experiments in which cardiometer and blood pressure readings were obtained, the animals were morphinized, etherized and the chest opened under artificial respiration. The cardiometer was of the glass bulb type with a perforated rubber membrane to fit around the auriculo-ventricular groove. Care was used in its application so that the blood flow into the ventricle might not be obstructed. Systolic and diastolic pressures were obtained with maximal and minimal valves. Venous pressure was determined by inserting a sound into the vena cava through the femoral vein and balancing the pressure against a manometer filled with salt solution.

X-ray examination after hemorrhage. According to the known reactions to hemorrhage which have already been mentioned, it has been generally thought that following a rapid loss of blood there is at once, or certainly within a few seconds, a peripheral vasoconstriction and an increased heart rate. Since the blood volume is decreased these reactions would seem to necessitate a decreased cardiac output per beat. This could be brought about by diminution of systole, or as more naturally and usually supposed, by a decrease in diastolic size, that is in cardiac filling.

X-ray photographs of the heart before and after hemorrhage show, however, contrary to the opinion just expressed, that the diastolic size of the heart is maintained even after the loss of large quantities of blood. In twenty-one experiments, each with from two to six hemorrhages, the heart did not decrease more than 5 per cent, a figure we have allowed for unavoidable variations in the method, until the loss of blood on an average equaled 2 per cent of the body weight. The lowest figure was 1.2 per cent and the highest 2.9 per cent. In the dog this represents a loss of from 11 to 28 per cent of the blood volume. In table 1 may be seen a summary of nine of these experiments. The others were quite similar but have not been included for the sake of space. Figures 1 and 2 present the data of two of the experiments not included in table 1. The steady decrease in blood volume is sharply contrasted with the sudden reduction in diastolic heart size which occurs when about 20 per cent of the blood is lost, and which would seem to indicate the breakdown of some protective mechanism.

The advantage to the heart in retaining its diastolic size is evident in light of the work of Patterson, Piper and Starling (4) and Gesell (5), who have shown that, as in the case of skeletal muscle, the efficiency of cardiac contraction depends on the length of the muscle fibers.

Just how the heart after hemorrhage secures a venous return adequate and under sufficient pressure to maintain the normal distention of the ventricle is by no means a simple problem. It is not due to any change in pulse rate. It is of course well known that hemorrhage increases heart rates, but this does not occur until considerable blood is lost, a fact not always observed in the literature. In our experiments it will be seen in the curves and in table 1 that there was usually no significant change in heart rate until the sudden decrease in cardiac size occurred. To favor the heart in retaining its diastolic size the rate would have to decrease along with the loss of blood. This did not occur in our experiments, although experiments H 13 and H 27 may at first glance seem to be exceptions. These experiments show a gradual reduction of rate due to the passing off of atropine. The rates, however, came to a standstill during the last hemorrhages just at the time when changes would have been most marked if this were a mechanism enabling the heart by complete filling to retain its diastolic size.

After the loss of large amounts of blood the pulse is greatly accelerated. This acceleration often occurs with the marked decrease in diastolic size of the heart, as may be seen in figures 1 and 2. In other experiments, however, such as H 9, H 13, H 14 and H 27, there are decreases of cardiac size unassociated with any increase in pulse rate. It would seem that the sharp decrease in size of the cardiac silhouette area was associated with some phenomenon other than changes in heart rate.

It might be said that the heart retains its size after hemorrhage due to the inflow of tissue juices which at once restores the blood volume. That hemorrhage is followed by a dilution of the blood and that this may be detected even at the end of a short hemorrhage is an old observation dating from the time of Vierordt (6) or even earlier. Textbook statements often lead us to believe that in a few minutes this inflow entirely replaces the lost fluid. Such however we do not believe to be the case. The very extensive literature on this subject has few examples of significant dilutions under 20 minutes. Scott (7) for example, found in one dog that 59 minutes after bleeding 206 cc. the hemoglobin indicated return of only 23 cc. from the tissue fluid. The quickest return was 71 cc. in 20 minutes after a hemorrhage of 212 cc. These were 8 and 7 kilo dogs respectively. Richet, Brodin and Saint-Girons (8) have recently studied the effect of hemorrhage on the specific gravity of the blood. They found that after hemorrhages of 125 cc. in dogs averaging about 15 kilos the specific gravity at once fell but became stable in

TABLE 1

Showing the effect of hemorrhages of varying degrees on heart rate, blood pressure and diastolic size of the heart. The amount bled is expressed in cubic centimeters and in per cent of body weight. Corrections have not been made for the specific gravity of blood. The area of the heart is expressed in square centimeters

EXPERIMENT NUMBER	WEIGHT IN KILOS	PROCEDURE	PER CENT BODY WEIGHT	HEART RATE	BLOOD PRESSURE	DIASTOLIC SIZE
H 8	5.0	Normal		120	126	52.9
		Bled 75 cc.	1.5	120	122	50.9
		Bled 50 cc.	1.0	220	110	42.4
H 9	4.6	Normal		82	130	46.0
		Bled 75 cc.	1.6	80	134	45.1
		Bled 60 cc.	1.3	82	116	35.3
H 11	4.2	Normal		194	110	35.7
		Bled 75 cc.	1.8	194	114	34.2
		Bled 50 cc.	1.2	204	106	30.8
H 12	5.0	Normal		216	180	39.7
		Bled 160 cc.	3.2	192	168	36.8
H 13	5.5	Normal		156	170	50.8
		Bled 50 cc.	0.9	144	160	50.7
		Bled 50 cc.	0.9	132	150	50.8
		Bled 25 cc.	0.45	120	140	46.2
H 14	5.7	Normal		168	156	48.9
		Bled 50 cc.	0.09	162	160	48.3
		Bled 50 cc.		144	160	43.2
H 27	3.5	Normal		164		37.0
		Bled 23 cc.	0.6	150		37.0
		Bled 23 cc.	0.6	144		36.5
		Bled 20 cc.	0.57	144		33.5
H 37	4.8	Normal		88		32.3
		Bled 25 cc.	0.5	88		32.0
		Bled 25 cc.	0.5	96		32.3
		Bled 25 cc.	0.5	96		31.4
		Bled 25 cc.	0.5	120		26.1
H 35	4.7	Normal		96	94	38.2
		Bled 25 cc.	0.5	90	96	40.1
		Bled 25 cc.	0.5	93	110	39.8
		Bled 25 cc.	0.5	108	94	37.8
		Bled 25 cc.	0.5	120	112	32.5

about 15 minutes. A 10 per cent loss of blood reduced the specific gravity from 1.056 to 1.054, which indicates a dilution of about 4 per cent.

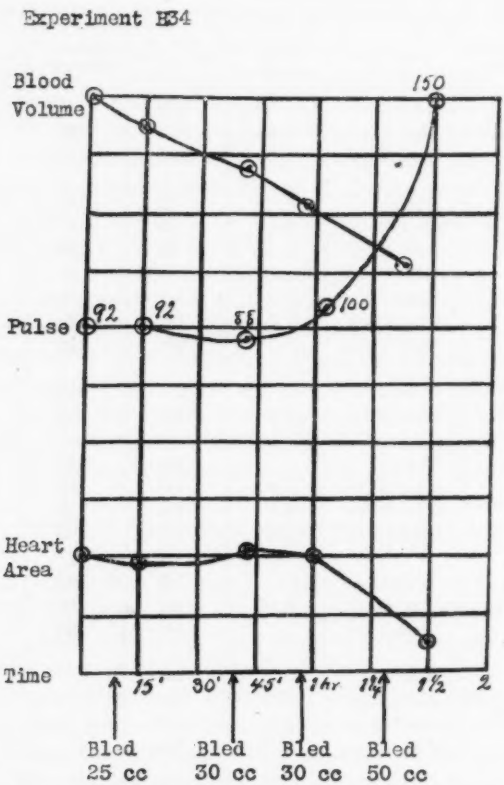

Fig. 1. Curves showing the changes in blood volume, pulse rate and heart size which occurred after four successive hemorrhages. The first determinations are the normals. Changes from normal are in per cents, 10 per cent for each line unless the actual figures are inserted. The maintenance of heart size after a loss of nearly 20 per cent of the blood volume is to be noted.

In a number of our experiments we have followed the dilution of the blood by means of hemoglobin and specific gravity determinations. Figure 2 shows that in one experiment the hemoglobin had been reduced only 6 per cent at a time when the blood volume had been decreased

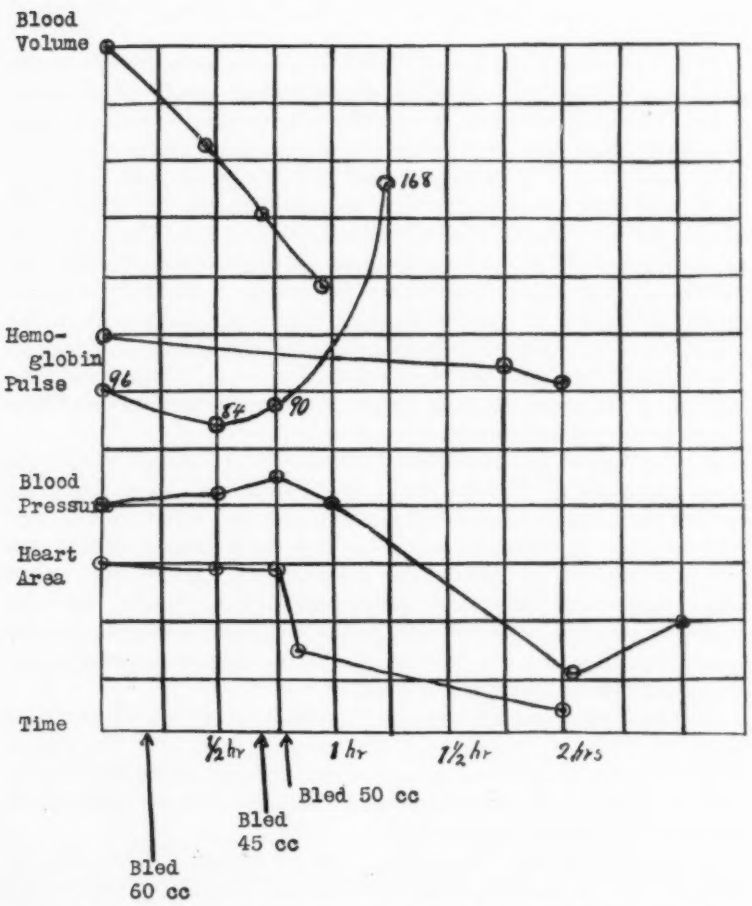

Fig. 2. Curves showing the changes in blood volume, hemoglobin, pulse rate, blood pressure and heart size after three successive hemorrhages. Determinations are plotted as in figure 1. The maintenance of heart size after hemorrhages amounting to nearly 30 per cent of the blood volume is very striking. At this time the pulse rate was within 6 beats of normal and the blood pressure had increased 5 per cent. The third bleeding destroyed the compensating mechanism and the diastolic heart size decreased at once.

40 per cent. Other experiments showed somewhat greater dilutions but in none was the inflow of tissue fluid during the period of the experiment sufficient to restore the blood volume to anything like its normal value.

In agreement with Richet we have found that specific gravity determinations give evidence of dilution earlier than can be obtained by hemoglobin readings. For determining specific gravity we have used Hammerschlag's method. In three experiments in which the hemorrhage equaled 15 to 25 per cent of the blood volume, the specific gravity during the time necessary for x-ray examinations fell only 0.002, that is from 1.054 to 1.052. This would indicate that less than 5 per cent of the blood volume had been replaced by tissue fluids. Neither the literature nor our own observations would seem to justify attributing to the absorption of tissue fluid more than a secondary part in the maintenance of the cardiac diastolic size after hemorrhage. It may be worth noting that in rabbits blood volume is more quickly restored than in dogs, a conclusion we have arrived at from the literature and confirmed by several experiments of our own.

If after hemorrhages amounting to 2 per cent of the body weight the heart maintains its diastolic size it would seem that up to this point cardiac filling was not interfered with and that the output per beat was still normal. It might, however, be that the extent of systole was reduced and that, undetected by diastolic x-ray picture, the output had actually been falling with the decreased blood volume. In order to investigate this point we studied the effects of hemorrhage in the anesthetized dog with the chest open by means of the cardio-plethysmograph and blood pressure manometers.

Cardio-plethysmographic and blood pressure records after hemorrhage. In tables 2 and 3 are presented the complete data from two experiments showing the effect of successive hemorrhages on cardiac output, venous pressure and arterial pressure. Eight other experiments have been made with quite similar results.

It will be seen from these tables that during and immediately following small hemorrhages amounting to less than 1 per cent of the body weight, the cardiac output per minute is always, and systolic, diastolic and pulse pressures are usually, slightly decreased. Within a few minutes or even less, however, recovery of the normal is generally complete.

In this series of eight experiments the cardiac output per minute never failed to be reduced immediately after a small hemorrhage.

The reduction, so long as the blood lost was under 1 per cent of the body weight, was not great. It was brought about by a decrease in output per beat rather than by changes in heart rate. The limited

TABLE 2

Data from experiment 5 showing the effect of five successive hemorrhages

TIME	PROCEDURE	PULSE RATE	VENOUS PRESSURE	CARDIAC OUTPUT		ARTERIAL BLOOD PRESSURES			
				Per minute	Per beat	Systolic	Diastolic	Mean	Pulse pressure
2.39	Normal	114	8.0	2780	24.4	119	56	70	63
2.45	After bleeding 0.2 per cent of body weight	102	7.75	2505	24.6	120	56	68	64
2.48	Three minutes after bleeding	102	7.75	2794	27.3	127	62	74	65
2.50	Second bleeding total of 0.40 per cent body weight	114	7.5	2540	22.8	124	59	73	65
2.53	Three minutes after second bleeding	114	7.0	2979	26.1	131	63	75	68
2.56	Third bleeding total of 0.67 per cent body weight	114	7.0	2547	22.3	114	54	72	60
3.07	Eleven minutes after third bleeding	120	8.0	2479	20.6	140	76	92	64
3.22	Twenty-six minutes after third bleeding	120	8.0	2492	20.8	108	49	63	58
3.30	Fourth bleeding total of 0.8 per cent body weight	156	6.75	968	6.2	101	61	72	40
3.38	Eight minutes after fourth bleeding	162	6.25	1160	7.2	107	63	71	44
3.41	Fifth bleeding total of 1.1 per cent body weight	162	6.25	1071	6.9	88	48	58	40
3.44	Three minutes after fifth bleeding	162	6.25	1084	6.9	85	50	60	35

extent of the reduction, amounting only to 2 or 3 cc. per beat, together with the rapid recovery, prevented us from detecting this change by means of the x-ray. In a very few minutes the minute output returned not only to normal but usually well above.

TABLE 3

Data from experiment 7 showing the effect of nine successive hemorrhages

TIME	PROCEDURE	PULSE RATE	VENOUS PRESSURE	CARDIAC OUTPUT		ARTERIAL BLOOD PRESSURE			
				Per minute	Per beat	Systolic	Diastolic	Mean	Pulse pressure
2.55	Normal	138	9.5	1642	11.9	95	50	58	45
3.02	Bled 0.13 per cent body weight	138	9.5	1371	9.9	93	53	60	40
3.06	Four minutes later	138	10.0	1820	13.2	115	67	72	48
3.07	Second bleeding total of 0.28 per cent body weight	138	10.25	1629	11.8	117	63	73	54
3.09	Two minutes later	132	10.25	1679	12.7	109	59	72	50
3.14	Third bleeding, total of 0.43 per cent body weight	144	9.75	1442	10.0	107	65	68	42
3.18	Four minutes later	144	10.5	1771	12.3	119	73	83	46
3.24	Ten minutes after third hemorrhage	150	9.5	1841	12.2	118	79	86	39
3.34	Fourth bleeding total of 0.60 per cent body weight	144	9.25	1504	10.4	120	66	78	54
3.39	Five minutes later	156	9.5	1722	11.1	123	70	83	53
3.41	Fifth bleeding total of 0.75 per cent body weight	150	9.5	1614	10.7	118	66	74	52
3.45	Four minutes later	144	8.75	1629	11.3	125	70	80	55
3.46	Sixth bleeding total of 0.92 per cent body weight	150	8.75	1387	9.3	112	64	71	58
3.51	Five minutes later	144	8.5	1295	8.6	123	68	82	55
3.54	Seventh bleeding of 1.2 per cent body weight	144	8.0	1283	8.9	102	65	71	37
4.02	Eight minutes later	144	8.25	1216	8.4	105	64	75	41
4.06	Eighth bleeding 1.5 per cent body weight	150	8.5	829	5.5	98	52	68	46
4.11	Five minutes later	150	8.5	989	6.6	102	56	65	46
4.13	Ninth bleeding 1.8 per cent body weight	150	7.75	622	4.4	80	44	52	36
4.21	Eight minutes later	150	7.5	730	4.8	88	46	60	42
4.41	Twenty-eight minutes after ninth hemorrhage	150	7.75	725	4.8	76	28	48	48

Arterial blood pressures were somewhat more inconstant than the volume outputs. The result of the first bleedings was often a tendency to raise systolic pressure either at once or during the next few minutes. Both of the experiments submitted illustrate this point. Later there was a slight fall of systolic pressure with a prompt recovery. Venous pressure showed no significant changes.

When the amount of bleeding averaged from 0.75 to 1 per cent of the body weight the usual slight reduction in output appeared after the hemorrhage but the recovery was not as complete as previously. For example, in experiment 5, table 2, 3 minutes after the second bleeding the minute output exceeded the normal by nearly 200 cc., but after the third bleeding the recovery was not quite to normal.

When the amount of blood drawn exceeded about 1.2 per cent of the body weight the cardio-vascular reactions differed from those above described. The decrease in output per minute and per beat became particularly marked. The systolic and pulse pressures were considerably lowered. The diastolic and mean pressures, however, usually held up near normal until a later bleeding. Venous pressure was permanently lowered. These changes may be noted as the result of the fourth bleeding in table 2 and the eighth bleeding in table 3.

It would seem that the cardio-vascular reactions to hemorrhage as determined particularly by output fell into three stages. First after bleedings not exceeding on the average 0.7 per cent of the body weight there is quick recovery with over-compensation, that is an actual increase in output. Second, after bleeding from 0.7 to 1 per cent of the body weight there is a return of the output to normal but the rebound above normal, so marked in the first stage, is lacking. One is tempted to assume that some compensating mechanism has reached its limit and that this is a critical stage. Third, after hemorrhages amounting to 1.2 per cent of the body weight or more there is failure to increase the output anywhere near to normal. These figures are of course only approximate, varying in different animals, but the stages themselves were apparent in all the experiments.

The effect of hemorrhage on the capillaries and venules. So far we have shown that, except for a very brief period immediately after bleeding, the heart may retain its diastolic size and keep up its usual minute volume output until there is a total hemorrhage of about 2 per cent of the body weight in the intact animal under morphia, and about 1.2 per cent when the heart is exposed under anesthesia and artificial respiration. Since in the dog the total amount of blood is nearly 10 per cent

of the body weight (9), (10), these figures indicate that in the intact animal during a reduction of approximately 20 per cent in blood volume the heart may still keep up its normal output. To do this the right heart must continue to receive its usual supply of blood, for the systolic output certainly depends directly on the inflow during diastole. The problem resolves itself into finding an explanation for the adequate blood supply to the right heart. That the body fluids cannot serve as a source in the time required we have already shown. Theoretically the conditions might be met if the velocity of the venous return were sufficiently augmented. For this, however, there is no evidence, since the venous pressure remained unchanged. The same fact would seem to preclude a decrease in the capacity of the pulmonary bed sufficient to compensate for the hemorrhage. The portal circulation with its double resistance is particularly fitted to serve as a reservoir for the venae cavae, as pointed out by Krogh (11), but constriction of arterioles is necessary to render this supply available. The failure of systolic blood pressure uniformly to increase after the bleedings seems to exclude this explanation. The recent work of Hooker (12) on the functional activity of the venules and capillaries has called our attention to a possible solution of the problem. If there are venules or capillaries containing blood, but not in the active course of the circulation, a constriction of these vessels would add fluid and tend to maintain the effective circulation. Cardiac output, after a brief period for the readjustment, might be maintained so long as the content of stagnant venules and capillaries sufficed to meet the loss from hemorrhage. That there are such capillaries and venules we know from the work of Roy and Brown (13) and Danzer and Hooker (14). The latter discuss fully the literature of this very interesting subject.

To find if there is any evidence for such a hypothesis, we have observed the venules and capillaries in the ears of dogs before, during and after hemorrhage. For this purpose we have used the method of illumination described by Hooker (12). Three animals have been studied. Dog 1 weighed 3.65 kilos. After bleeding 100 cc. or 2.7 per cent of his body weight the blood vessels slowly decreased during the next 3 minutes. That this was due to the hemorrhage could scarcely be doubted for the flow had continued for sometime previously. Dog 2 weighed 8 kilos. He was bled 100 cc. with no observable effect. On a second bleeding, when the hemorrhage had reached a total of 160 cc., there was an almost instantaneous whitening of the ear caused by the constriction of the venules and capillaries, both of those that had been

very active, and those that had been less so or entirely stagnant. There was thus a noticeable effect on the blood vessels when the bleeding equaled 2 per cent of the body weight. Dog 3 weighed 10.8 kilos and was bled 216 cc. Photomicrographs were taken immediately before and after the hemorrhage. These are reproduced in figure 3. The constriction of the venules and capillaries is very striking.

These experiments are clear proof that at a certain stage of hemorrhage there is some kind of a circulatory reaction resulting in the

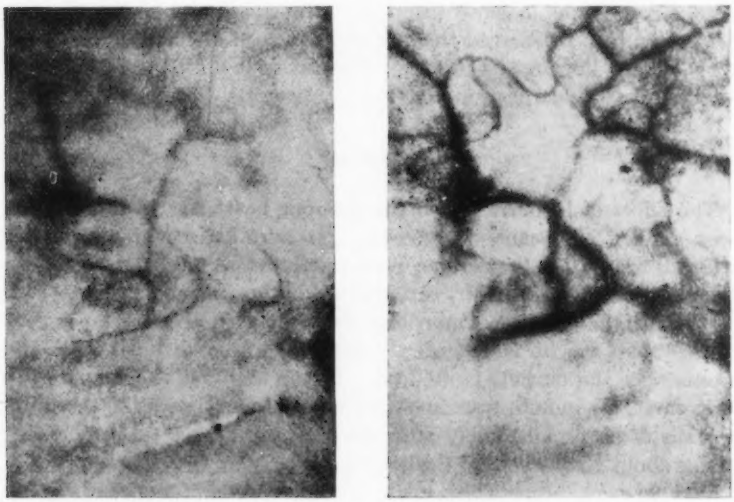

Fig. 3. Photomicrographs of the surface of a dog's ear before and after a hemorrhage of 215 cc. or 2 per cent of the body weight. Magnification 90 ×. Plates not retouched.

constriction of the venules and capillaries in the skin. All the indications are that the response is active and not passive. Whether or not it is brought about through nervous connections has not been investigated. The most obvious purpose served by this reaction would be to furnish an adequate supply of blood for the venous return to the right heart.

It is realized that the constriction of vessels in the skin could not make up for a very large hemorrhage since only some 2 or 3 per cent of the total blood volume is normally found at the surface of the body.

What we have seen is probably the last stage in the body's attempt to keep up the effective circulation. At first the stagnant vessels in the muscles or organs of the splanchnic area are probably called upon. Later when the circulation is at the breaking point, as it is when the bleeding equals 2 per cent of the body weight, venules and capillaries are constricted in widespread areas. In a word, the fact that at a certain stage of hemorrhage the venules and capillaries are seen to constrict justifies the hypothesis that there may have been initial constriction, particularly of stagnant vessels, which would thereby add a sufficient volume of blood to preserve the status quo of the effective circulation. It is believed that by some such means as this the heart is enabled to keep up its minute volume output until the hemorrhage reaches relatively large proportions.

SUMMARY

The effect of hemorrhage on the diastolic heart size of the dog has been studied by means of the x-ray. In anesthetized animals under artificial respiration the cardiac output and venous pressures have been determined after bleeding by cardiometers and manometers.

In the intact animal hemorrhage amounting to about 2.1 per cent of the body weight is necessary before the diastolic heart size, and presumably the output, is reduced. In the anesthetized animal with open chest, the minute volume output is maintained, with the exception of a slight drop immediately after bleeding, until the total hemorrhage equals about 1.2 per cent of the body weight.

Various mechanisms which might account for maintenance of cardiac output under these conditions are discussed. The only satisfactory explanation seems to be that the effective circulation is kept up by constriction of venules and capillaries, particularly those which have been more or less stagnant. Evidence in support of this idea is submitted. When hemorrhage in the intact animal reaches about 2 per cent of the body weight, the venules and capillaries of the ear may be seen markedly to constrict. Since such a mechanism is thus shown to exist, it becomes probable that it may have been operating in various parts of the body in earlier stages of the hemorrhage and that in this way there was provided a constant venous return and cardiac output even though the blood volume was decreased.

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FURTHER EXPERIMENTS ON THE ACTIVATION OF MUSCLE CATALASE BY LIVER[1]

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In 1918 I published an account of some observations on catalase in which it was shown that when liver and muscle act together on hydrogen peroxide, the amount of gas given off is in excess of the sum of the two acting separately (1). Loevenhart (2), in a similar observation in 1905, attributed it to a neutralizing effect on the retarding action of the acid, and argued against the presence of a kinase. About the same time Battelli and Stern (3) described a philocatalase which had the property of antagonizing anticatalase and of regenerating catalase. They also described an "activator" of philocatalase. Some earlier experiments of my own seemed to show that muscle acted as well in acid as in neutral peroxide, and this, taken with other reasons, led me to the tentative suggestion that there might be an accelerator secreted by the liver. At this point the work was brought to an abrupt termination by absence from the University and the question of an accelerator was left for the future. Upon my return I again took up the problem, but was unable to get results that were consistent. Only occasionally could acceleration be obtained in acid H_2O_2. Meanwhile Takeda (4) pointed out that the acceleration in neutral peroxide was absent in many cases. He also found that acceleration occurred in acid H_2O_2 when liver was combined with boiled muscle, egg white or peptone solution. Takeda suggests that the accelerating effect is due to some organic substance contained in the tissue; possibly a "protective colloid."

Having convinced myself that the hypothesis of a liver hormone is untenable, the following results are offered as a somewhat more definite explanation of the phenomenon than those hitherto suggested.

[1] The expenses of this research were paid out of a grant from the Research Board of the University of California.

Method. Granted an acceleration of catalytic activity when liver and muscle are placed in acid H_2O_2, and no acceleration when placed in neutral H_2O_2, it does not follow that there is no accelerator secreted by the liver. It may be one which acts in an acid medium and not in a neutral. Obviously the thing to do is to separate the two if possible, and with that in view catalase was prepared from rabbits' liver and muscle by a method which is a combination of that of Battelli and Stern (5) for catalase, and Van Slyke and Cullen (6) for urease. (My attention was called to the latter by my colleague, C. L. A. Schmidt of the Department of Biochemistry.) The rabbits were perfused through the aorta with m/6 NaCl until the fluid ran white from the veins. The tissue was reduced to a pulp in a meat grinder and shaken for an hour or so with twice its volume of distilled water. It was then strained through linen in a press and the pulp subjected to a second extraction with its own volume of distilled water, the two watery extracts were added together and poured slowly into "a volume of acetone so large that the enzyme undergoes practically instant dehydration." (Van Slyke and Cullen state that the volume of acetone should not be less than ten times that of the water extract, but I have obtained excellent preparations of catalase with three or four volumes.) The dense precipitate was filtered through hardened filter paper. After evaporating the acetone from the precipitate by a current of air, the precipitate was mixed with two volumes of distilled water and either shaken for an hour or two or allowed to stand for several hours. It was then filtered, and the filtrate precipitated with acetone as before. After filtering through hardened filter paper, the precipitate was dried in a vacuum over sulphuric acid, and pulverized in a mortar. This gives a catalase impure of course, which is very active. Twenty milligrams of liver catalase thus prepared from rabbit's liver gave off 69 cc. of oxygen from neutral hydrogen peroxide in 10 minutes. Twenty milligrams of muscle catalase prepared from the same rabbit gave 12 cc. of oxygen in 10 minutes. Two hundred grams of liver yielded about 3 grams of catalase, while 200 grams of muscle gave but 0.9 gram. The amounts vary, however, with the individual.

Other methods have been tried, such as precipitation with ether and with alcohol, but the above method has yielded a more soluble preparation in my hands. Incidentally it may be said that precipitation with ether does not destroy the catalase, although Burge (7) states that liquid ether will destroy catalase very quickly.

Results. When liver catalase and muscle catalase act together on acid or neutral H_2O_2 there is practically no acceleration. The slight acceleration shown in table 1 is within the experimental error of the method used, as Becht (8) has pointed out. The catalase was prepared as described in the text. The volume of oxygen is reduced to 0° and

TABLE 1

	SUBSTANCE	QUANTITY	H_2O_2 REACTION	O_2	VOLUME INCREASE	ACCELERATION
		grams		*cc.*		*per cent*
1	Muscle catalase	0.02		6		
	Liver	0.02	Acid	23	+1	+3.0
	Muscle catalase 0.02 gram and liver catalase	0.02		30		
2	Muscle catalase	0.02		6		
	Liver	0.02	Acid	27	0	0
	Muscle catalase 0.02 gram and liver catalase	0.02		33		
3	Muscle catalase	0.02		4		
	Liver	0.02	Acid	14	−1	−5.5
	Muscle catalase 0.02 gram and liver catalase	0.02		17		
4	Muscle catalase	0.02		11		
	Liver	0.02	Neutral	69	+3	+4.0
	Muscle catalase 0.02 gram and liver catalase	0.02		83		
5	Muscle catalase	0.02		4		
	Liver	0.02	Neutral	47	+2	+4.0
	Muscle catalase 0.02 gram and liver catalase	0.02		53		
6	Muscle catalase	0.02		6		
	Liver	0.02	Neutral	56	+2	+3.0
	Muscle catalase 0.02 gram and liver catalase	0.02		64		

760 mm. Hg. taking the nearest whole number. The H_2O_2 was neutralized with N/10 NaOH until it had a pH 7 by the Clark and Lubs scale. In all cases the time is 10 minutes' shaking. The temperature was constant for any given series.

At first sight these results seem to indicate that something in the nature of an accelerator had been removed from the liver catalase; but

TABLE 2

	SUBSTANCE	QUANTITY	H_2O_2 INITIAL pH	O_2	VOLUME INCREASE	ACCELERATION	H_2O_2 FINAL pH
				cc.		*per cent*	
1	Muscle	0.5 gm.	2.4	6	+17	+113	4.5
	Liver	0.1 gm.	2.4	9			2.4
	Muscle 0.5 gram and liver	0.1 gm.	2.4	32			4.5
2	Muscle	0.5 gm.	2.4	6			4.5
	Egg white (boiled)	0.5 gm.	2.4	0	+1	+17	4.5
	Muscle 0.5 gram and egg white	0.5 gm.	2.4	7			4.8
3	Muscle	0.5 gm.	2.4	6			4.5
	Serum (rabbit)	0.5 cc.	2.4	0	+2	+33	4.6
	Muscle 0.5 gram and serum	0.5 cc.	2.4	8			4.8
4	Muscle	0.5 gm.	2.4	6			4.5
	Muscle	0.5 gm.	2.4	7	+6	+46	4.5
	Muscle	1.0 gm.	2.4	19			4.8
5	Muscle	0.5 gm.	2.4	9			4.5
	Liver catalase	0.02 gm.	2.4	6	+7	+47	2.4
	Muscle 0.5 gram and liver catalase	0.02 gm.	2.4	22			4.6
6	Muscle (boiled)	0.5 gm.	2.4	0			3.6
	Liver catalase	0.02 gm.	2.4	5	+4	+80	2.4
	Muscle 0.5 gram and liver catalase	0.02 gm.	2.4	9			3.6
7	Liver	0.1 gm.	2.4	16			2.4
	Egg white (boiled)	0.5 gm.	2.4	0	+4	+25	4.5
	Liver 0.1 gram and egg white	0.5 gm.	2.4	20			4.5
8	Liver	0.1 gm.	2.4	15			2.4
	Serum (rabbit)	0.5 cc.	2.4	0	+8	+53	4.6
	Liver 0.1 gram and serum	0.5 cc.	2.4	23			4.8
9	Liver	0.1 gm.	2.4	14			2.4
	Liver	0.1 gm.	2.4	14	+4	+14	2.4
	Liver	0.2 gm.	2.4	32			2.6
10	Muscle catalase	0.02 gm.	2.4	8			2.4
	Liver (fresh)	0.1 gm.	2.4	58	0	0	2.4
	Muscle catalase 0.02 gram and liver	0.1 gm.	2.4	66			2.4

TABLE 2—*Concluded*

	SUBSTANCE	QUANTITY	H_2O_2 INITIAL pH	O_2	VOLUME INCREASE	ACCELERATION	H_2O_2 FINAL pH
				cc.		*per cent*	
11	Casein (neutral)	0.5 gm.	2.4	0			3.6
	Liver catalase	0.02 gm.	2.4	7	0	0	2.4
	Casein 0.5 gram and liver catalase	0.02 gm.	2.4	14			3.6
12	*Na caseinate	0.5 gm.	2.4	0			5.6
	Liver catalase	0.02 gm.	2.4	5	+60	+1200	2.4
	Na caseinate 0.5 gram and liver catalase	0.02 gm.	2.4	65			5.6

* The Na caseinate was made by dissolving 1 gm. casein in 8 cc. of $\frac{N}{10}$ NaOH. It remains colorless on the addition of phenolphthalein (9).

referring to table 2 it will be seen that liver catalase plus muscle tissue gives acceleration. Loevenhart found acceleration with boiled liver, and Takeda used boiled muscle and even egg white with similar results. I have found acceleration not only with boiled white of egg, but also with blood fibrin and with gelatin. Now these substances are all proteins, and therefore attention was turned to their effect on the H-ion concentration of the peroxide. The hydrogen ion concentration of the peroxide was determined by Clark and Lubs scale very kindly prepared for me by Mr. S. B. Randall of the Department of Pathology. The substance was then added and after 10 minutes' shaking the pH of the solution again determined. Table 2 gives the results.

Note that in all cases of acceleration there is a shifting of the pH toward the neutral point. Where the muscle acts alone the pH of the solution is changed from 2.4 to 4.5, while the liver, on account of its small bulk does not perceptibly change the H ion concentration. When the two are added together, however, the liver catalase acts on H_2O_2 with a pH of 4.5 instead of 2.4 and therefore more oxygen is given off. That this is so is shown by the following:

0.02 gram liver catalase, H_2O_2 pH 2.4 gave 3 cc. O_2
0.02 gram liver catalase, H_2O_2 pH 3.8 gave 8 cc. O_2
0.02 gram liver catalase, H_2O_2 pH 5.0 gave 38 cc. O_2
0.02 gram liver catalase, H_2O_2 pH 7.3 gave 48 cc. O_2

Liver catalase was used because it does not of itself perceptibly change the pH of the H_2O_2 in the small quantities used.

CONCLUSION

It would seem then, that we must abandon the hypothesis of a liver hormone and conclude that at least one of the factors of the acceleration is a change in pH of the H_2O_2, brought about by the action of the proteins and possibly salts also. If we take Loevenhart's "neutralization" and Takeda's "protective action" in a broad sense, they are in agreement with the above. Whether this is a "buffer" action of the proteins or an adsorption phenomenon is not within the scope of this paper to determine.

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THE SOURCE OF DIASTASES OF THE BLOOD

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In doing some work on chloroform anesthesia we found certain changes in the blood diastases. Knowing that chloroform injures liver cells, the question arose as to whether the blood diastases might not come chiefly from the liver, instead of the pancreas, as Gould and Carlson's work indicates (1). It was thought possible that measurements of changes in the diastase content of the blood after pancreas operation and administration of ether and chloroform might aid in determining the source of the starch-splitting enzymes of the blood serum.

Experimental work. The determination of the serum diastases was made according to the method devised by McGuigan and Von Hess (2) except the starch solution used was $\frac{1}{2}$ per cent instead of 1 per cent. The essential of the method is the incubation of a measured volume of $\frac{1}{2}$ per cent soluble starch solution with a measured amount of serum until no color is produced by the mixing of 1 cc. of dilute iodine-potassium iodide solution and one drop of incubated mixture. The results are expressed in minutes required for the disappearance of the color reaction.

Serums were prepared from a number of normal dogs and the diastase contents determined. The results are given in table 1.

A group of animals was operated on to remove all of the pancreas except the tip of the tail which was implanted with its own blood supply under the skin of the abdomen. Determinations were made on these animals 3 days in succession after they had well recovered from the operation. The average results for each animal are given in table 1.

After observations were completed on the animals from which the pancreas had been partially removed, the remaining fragment of pancreas was taken away. After several days, a series of determinations on the diastase content of the serums of these dogs was made. The

results are given in table 1, the average of several days' observations being taken.

The immediate effects of anesthesia on the blood diastases were determined on several groups of dogs. A group of animals with healed partial pancreatectomies was given 15 minutes of ether anesthesia. The blood diastases were measured before and after this procedure. A second group of dogs with healed complete pancreatectomies was subjected to the same tests. A third group of dogs was given chloroform and the diastases measured before and after 15 minutes of anesthesia. These were normal animals. A group of partially pancreatectomized

TABLE 1

The effect of partial and complete removal of the pancreas on blood diastases (expressed in minutes)

NORMAL DOGS	DOGS PARTIALLY PANCREATECTOMIZED	DOGS COMPLETELY PANCREATECTOMIZED
24	26.5	55.5
26*	9.0	30.5
18	24.2	34.0
21	14.5	27.0
20		26.6
27		27.0
21		
13		
36		
23		
22		
11		
13		
Average 21.1	18.5	33.4

* Dog had pancreatic cyst.

dogs was given chloroform and the tests made. The same was done with a series of completely pancreatectomized animals. The results of the above procedures are given in table 2.

The continued effect of anesthesia on the blood diastases of several groups of dogs was determined. After taking the blood and measuring the diastase concentration of a group of animals, they were anesthetized with ether for half an hour. The following day they were bled again and the diastase determined. Another group of normal dogs was subjected to the same treatment except chloroform was used in place of ether. A third group of normal dogs was given a fast of 2 days and

TABLE 2

Immediate effect of anesthesia and pancreatectomy on blood diastases

ANIMAL	PANCREAS OPERATION	ANESTHESIA	DIASTASE TIME (MINUTES)	
			Before anesthesia	After anesthesia
1	Partial removal	Ether	24	24
2	Partial removal	Ether	6	6
3	Partial removal	Ether	16	14
4	Partial removal	Ether	14	14
5	Partial removal	Ether	24	23
1	Complete removal	Ether	54	51
2	Complete removal	Ether	31	30
3	Complete removal	Ether	17	17
9	No removal	Chloroform	21	33
10	No removal	Chloroform	13	36
11	No removal	Chloroform	19	57
1	Partial removal	Chloroform	24	24
2	Partial removal	Chloroform	6	6
3	Partial removal	Chloroform	17	16
4	Partial removal	Chloroform	15	15
1	Complete removal	Chloroform	57	58
2	Complete removal	Chloroform	30	28
3	Complete removal	Chloroform	17	16

TABLE 3

Prolonged effect of anesthesia on blood diastases

ANIMAL	CONDITION	ANESTHETIC	DIASTASE TIME (MINUTES)	
			Before anesthesia	After anesthesia
12	Normal	Ether	5	6
13	Normal	Ether	23	19
14	Normal	Ether	22	15
15	Normal	Ether	11	11
16	Normal	Ether	13	13
17	Normal	Chloroform	14	21
18	Normal	Chloroform	20	26
19	Normal	Chloroform	15	24
20	Normal	Chloroform	19	25
21	Normal	Chloroform	16	23
22	Fasted	Chloroform	9	12
23	Fasted	Chloroform	20	31
24	Fasted	Chloroform	13	23
25	Fasted	Chloroform	16	34
26	Fasted	Chloroform	16	23
27	Fasted	Chloroform	15	30

tested in the same way as the second group. The results of these tests are given in table 3.

The group averages are given in table 4. The values for the immediate effect of ether on the diastases of normal dogs are taken from a previous article (3). The values here stated are half those in the article because in that work the starch solution was 1 per cent and in this ½ per cent.

TABLE 4

The average immediate and prolonged effects of anesthesia and pancreatectomy on blood diastases

PANCREAS	ANESTHETIC	OBSERVATION	DIASTASE TIME (MINUTES)		
			Before anesthesia	After anesthesia	Change
Normal	Ether	Immediate	20.5	20.0	−0.5
Partial removal	Ether	Immediate	16.8	16.2	−0.6
Complete removal	Ether	Immediate	34.0	32.6	−1.4
Normal	Chloroform	Immediate	17.6	42.0	+24.4
Partial removal	Chloroform	Immediate	15.5	15.2	−0.3
Complete removal	Chloroform	Immediate	34.8	34.0	−0.8
Normal	Ether	Prolonged	14.8	12.8	−2.0
Normal	Chloroform	Prolonged	16.8	23.8	+7.0
Fasted	Chloroform	Prolonged	14.8	25.5	+10.7

DISCUSSION

Schlessinger (4) removed the pancreas from dogs and found a decrease of blood disatases. He concluded that this enzyme originated in the pancreas.

Carlson and Luckhardt (5) removed the pancreas and obtained no change in blood diastases. They found a slight decrease in them after ether anesthesia.

Otten and Galloway (6) from extirpation experiments concluded that the pancreas is responsible directly or indirectly for a large portion of the diastases in the blood. They suggest the possibility that diastases in the liver cells may take some part in the splitting of glycogen that later passes into the blood stream.

Gould and Carlson (1) by operations on the pancreas have obtained results which lead them to conclude that ligation of the outlets of the pancreas increases the blood diastases and removal of the pancreas decreases the blood diastases.

Ross and McGuigan (3) made observations on the diastase changes brought on by ether anesthesia with variation in diet. Ether anesthesia caused no change in the blood diastases of dogs fed on either a meat or a mixed diet.

The work we desired to do required that the pancreas be removed completely from a number of dogs. The removal was done in two operations. The two-stage operation was for the purpose of producing the least amount of disturbance in the animal. Since the material was available, confirmatory tests were made to see the effect of the removal of the pancreas on the blood diastases. The results given in table 1 show the average diastase time as 21.1 minutes for normal animals, 18.5 minutes for partially pancreatectomized dogs and 33.4 minutes for dogs without any pancreas tissue. This confirms the findings of Schlessinger and Gould and Carlson, that the removal of the pancreas decreases the blood diastases.

The effect of ether anesthesia on the blood diastases of animals with varying amounts of pancreas was measured. The table of averages is number 4. Anesthesia of normal dogs with ether changed the diastase time from 20.5 to 20.0 minutes, of partially pancreatectomized dogs changed the diastase time from 16.8 minutes to 16.2 minutes, and dogs without any pancreas from 34.0 minutes to 32.6 minutes. The changes are considered negligible.

The effect of chloroform anesthesia on dogs with varying amounts of pancreas was distinctive. The average results are given in table 4. Normal dogs had their diastase time changed from 17.6 minutes to 42.0 minutes. This marked decrease in diastases in normal dogs due to chloroform anesthesia might be caused by either one or both of two things, i.e., liver injury, if the liver is a source of diasatases, and pancreas injury. It is commonly known that chloroform injures liver cells. Whipple and Sperry (7) found chloroform capable of producing considerable injury to the cells of the pancreas. Partially and completely pancreatectomized dogs did not have an appreciable change produced by chloroform anesthesia. This finding rules out the possibility that the liver injury was the cause of the change in blood diastases resulting from chloroform anesthesia and leaves us to conclude that the injury to the pancreas was the factor that reduced the diastases.

To test the influence of ether and chloroform further two groups of animals were given a half-hour anesthesia one day and the diastases measured before the anesthesia and the following day. One group of animals was anesthetized with ether, the other with chloroform. The

results are given in table 3. Since a fast preceding a chloroform anesthesia increases the injury to liver cells from the drug action (8), it is reasonable to suppose that the injury elsewhere, such as in the pancreas, would be likewise influenced. Therefore another group of dogs was subjected to a fast of 2 days and then given chloroform as before and diastase determinations made before the anesthesia and the following day. The results are given in table 3. The averages from table 3 are given in table 4. Ether anesthesia produced an average change from 14.8 minutes to 12.8 minutes in 24 hours. The change is negligible. A half-hour chloroform anesthesia changed the diastase time from 16.8 minutes to 23.8 minutes the following day, increasing the time 7 minutes. The group of animals fasting 2 days had their diastase time changed from 14.8 minutes to 25.5 minutes the following day, increasing the time 10.7 minutes.

Chloroform in these two groups of dogs again produced marked decreases in the blood diastases. In the group of animals subjected to a fast in which we would expect greater injury to the pancreas produced by chloroform, this drug brought about a greater decrease in the blood diastases than was the case with normal animals. These results again suggest that the pancreas is the chief source of the starch-splitting enzymes of the blood serum.

SUMMARY AND CONCLUSIONS

A group of 13 normal dogs was tested as to the diastatic power of their serums.

Groups of dogs which still retained part of the pancreas were tested 3 successive days as to the diastatic power of the blood. They were given ether anesthesia for 15 minutes and the diastases of the blood determined before and after. They were given chloroform anesthesia for 15 minutes and the same measurements were made.

Groups of dogs from which the pancreas had been completely removed were subjected to tests. The diastase content of the blood was determined. They were given 15 minutes of ether anesthesia and the diastases measured before and after the anesthesia. They were given 15 minutes of chloroform anesthesia and the same measurements made.

Three groups of animals were used to determine the effect of ether, chloroform and chloroform preceded by fasting upon the diastase content of the blood the following day. Anesthesia in each case was 30 minutes in length.

The results of the tests were as follows:

1. The removal of the pancreas decreased the blood diastases markedly.

2. Ether anesthesia had no effect on the diastases of either normal, partially pancreatectomized dogs or dogs with no pancreas.

3. Chloroform produced a marked fall in the blood diastases of normal dogs. It did not produce any appreciable change in animals whose pancreas had been partially or completely removed.

4. Half an hour of ether anesthesia produced no change in the diastases found present the following day.

5. Fasting before chloroform anesthesia increased the effect of the drug.

The preceding results with the findings of others quoted above lead us to conclude that the pancreas is practically the only source of the blood diastases.

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CHANGES IN THE CATALASE CONTENT DURING THE LIFE CYCLE

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It is known that oxidation is low in the unfertilized egg and that it increases after fertilization (1). It is also known that oxidation or metabolism is low in the newly born, that during youth it increases rapidly to a maximum, and gradually declines throughout adult life to become almost as low in extreme old age as at birth (2). There is much evidence that whenever oxidation is increased or decreased in animals as well as in plants there occurs a corresponding increase or decrease in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide (3).

The object of the present investigation was to determine if there is an increase or decrease in catalase corresponding with the increase or decrease in oxidation during the different phases of the life cycle enumerated above. The animals used were mice and Colorado potato beetles. After skinning the mice the entire animals were macerated vigorously in a mortar with a small amount of sand for 5 minutes. Whole beetles were macerated in a similar manner.

Catalase determinations were made by adding the macerated material to neutral hydrogen peroxide in a bottle and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content. The amount of macerated mouse was 1.0 gram and of the beetles 0.5 gram. Maceration for more than 5 minutes produced little or no effect on the amount of oxygen liberated by the material.

The results of the determinations are shown in figures 1 and 2. It may be seen in figure 1 that 0.5 gram of unfertilized beetle eggs liberated 18 cc. of oxygen from hydrogen peroxide in 10 minutes and a similar amount of fertilized eggs liberated 35 cc. The unfertilized eggs were removed from the body cavity of the beetles and washed in a 0.9 per cent solution of sodium chloride. The fertilized eggs were collected, soon after they were laid, from the leaves of the potato plant

and washed as were the unfertilized ones. The increase in catalase in the fertilized eggs is attributed to the stimulation of the egg by the spermatozoön to an increased output of this enzyme.

It may be seen further in figure 1 that 0.5 gram of newly hatched macerated larvae liberated 280 cc. of oxygen; quarter grown larvae, 800 cc.; half grown, 1250 cc.; three-quarter grown, 1725 cc.; pupae, 1800 cc.; adult beetles, 1750 cc.; and very old beetles, 900 cc. By com-

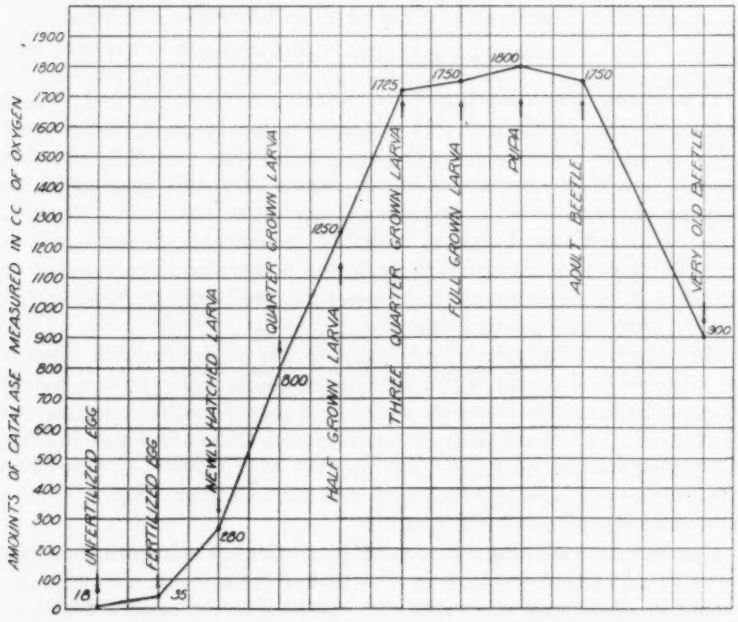

Fig. 1. The figures in the chart indicate amounts of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 gram of the macerated material.

paring these figures it may be seen that there were increasing amounts of oxygen liberated with the increase in age of the larvae and a marked decrease in catalase in the very old beetles.

In figure 2 are shown the amounts of oxygen liberated by macerated mice of different ages. It may be seen that 1.0 gram of newly born mice liberated 164 cc. of oxygen from hydrogen peroxide in 10 minutes; 24-hour mice, 190 cc.; 48-hour mice, 256 cc.; 144-hour mice, 288 cc.;

one-fourth grown mice, 435 cc.; one-half grown mice, 582 cc.; mother or adult mice, 715 cc. and very old mice, 400 cc. The very old mice were about 2 years old. By comparing these figures it may be seen that the amounts of catalase increased with an increase in age up to maturity, and that there was a decrease in catalase in the very old mice.

Determinations of the catalase content of young mice that had been deprived of food at birth were also made and it was found that the

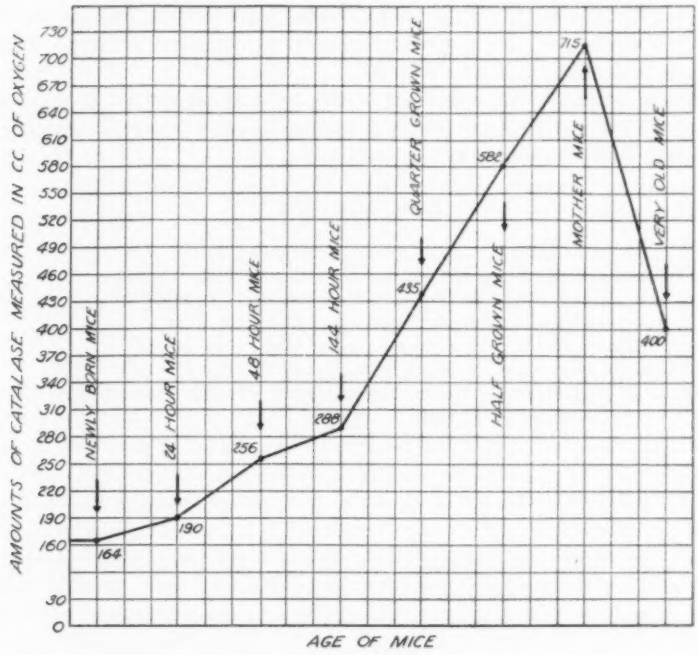

Fig. 2. The figures in the chart indicate amounts of oxygen liberated from hydrogen peroxide in ten minutes by 1.0 gram of the macerated material.

catalase content of these starved mice was not increased with an increase in age as was the case with the mice fed normally. We had already found that the ingestion of food greatly increased the blood catalase by stimulating the alimentary glands, particularly the liver, to an increased output of this enzyme. Hence the increase in catalase with increase in age is probably due to the stimulating effect of the food on the alimentary glands.

SUMMARY

The increased oxidation or metabolism with resulting development after fertilization is attributed to an increase in catalase brought about by the stimulation of the egg by the spermatozoön to an increased production of this enzyme.

The low metabolism or oxidation in the newly born is attributed to the low catalase content, while the intense metabolism characteristic of youth and adult life is attributed to the high catalase content due to the stimulation of the alimentary glands, particularly the liver, to an increased formation of this enzyme.

Similarly the low metabolism of the aged is attributed to the low catalase content brought about presumably by a lessened output of catalase from the alimentary glands.

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THE NUTRITIVE VALUE OF YEAST IN BREAD[1]

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Yeast has been used in bread making since the earliest times and continues as the great leavening agency, although sometimes substituted in this capacity by one of a variety of baking powders depending on the liberation of carbon dioxide from inorganic carbonates. There has also been some tendency of recent years to decrease the amount of yeast used in bread making as a matter of economy.

In view of the fact that bread is the chief of staple foods, it is of importance to know whether the yeast used in its preparation should be looked upon merely as a generator of carbon dioxide or whether yeast improves the nutritive value of the loaf. If, as seems clear, yeast must of itself be considered as a food, it is of importance to know in what way it supplements the other ingredients of bread and in what amounts it should be used to obtain a product of maximum nutritive value. Also we cannot afford to underestimate the possibility of yeasts ultimately replacing in the diet certain other important foodstuffs.

It has been known for a number of years that the ash of yeast is especially rich in the important element phosphorus, containing at the same time very considerable amounts of potassium and lesser amounts of other inorganic elements essential in nutrition. Yeast also contains a considerable amount of carbohydrate, including cellulose and gum-like substances and glycogen. It is high in protein and in a nucleic acid yielding purine and pyrimidine bases, a pentose, and phosphoric acid on hydrolysis.

That yeast contains an antineuritic substance was shown by Schaumann (1). Hopkins first showed yeast to contain a substance small amounts of which accelerated the growth of rats fed purified foods (2). These observations have been repeatedly confirmed and extended

[1] The expenses of this investigation were defrayed in part by a grant from Mrs. M. H. Henderson.

until it is now generally recognized that yeast is one of the richest and cheapest sources of water-soluble vitamine.

A number of experiments have been carried out to determine the extent of digestion and absorption by men and lower animals of the protein of yeast, values of from 82 to 98.4 per cent having been obtained where yeast was merely an added food. Yeast as a sole source of nitrogen was utilized by dogs to the extent of 66 to 76 per cent according to experiments of Schill, who found in spite of this relatively low coefficient of digestibility that the protein of yeast led to a greater nitrogen retention than was obtained with the usual diet of dog biscuits (3). That yeast could be used in human nutrition to replace from 10 to 30 per cent of the nitrogen of a mixed diet was shown in this laboratory by Hawk, Smith and Holder (4), who found biscuits prepared from yeast-containing flour were attractive in flavor, (in fact preferred by the subjects to ordinary white biscuits), and that the subjects gained more weight and showed a distinctly higher nitrogen retention on the yeast-containing diet. Osborne and Mendel found that rats could grow for over a year on a diet in which yeast was the sole source of protein as well as of water-soluble vitamine (5). Karr (6) found the feeding of yeast to dogs on a diet deficient in water-soluble vitamine and possibly protein to lead to increased nitrogen retention. He also found that addition of yeast to such a diet led to an increased consumption of food by such animals. Mattill and Conklin (7) found adolescent rats fed milk only did not thrive or reproduce, but the addition of yeast led to normal growth and partially successful reproduction.

Experimental procedure. The experimental procedure was simple. Young male albino rats, obtained from the rat colony of the Wistar Institute of Anatomy, were used as experimental subjects, and were divided into two groups of eleven rats each. The attempt was made to have the two groups as nearly alike as possible at the start of the experiment. The average weights for the two groups at the start were 60 and 61 grams respectively, as is shown in chart 3.

The rats in one group were fed white bread made to approximate closely standard home-made bread, while the rats in the other group were fed bread made with considerable extra yeast. The high yeast content was obtained by mixing 5 per cent of dried compressed yeast[2] with the flour and by using six times as much fresh yeast as was used

[2] Fleischmann's compressed yeast was used. This yeast was dried below 105°C., then powdered and mixed with the wheat flour.

in making the standard bread. The recipes used in making the two kinds of bread were as follows:

Extra yeast bread	*Standard bread*
2 quarts liquid ($\frac{1}{2}$ milk, $\frac{1}{2}$ water)	2½ quarts liquid ($\frac{1}{2}$ milk, $\frac{1}{2}$ water)
7½ lbs. flour	7½ lbs. flour
6 oz. dried yeast	6 oz. Crisco
6 oz. Crisco	3 teaspoons salt
3 teaspoons salt	3 cakes yeast
18 yeast cakes	3 teaspoons sugar
6 teaspoons sugar	

It is necessary to decrease liquid when using additional yeast. The dried yeast flour needs additional cake yeast to "raise" it.

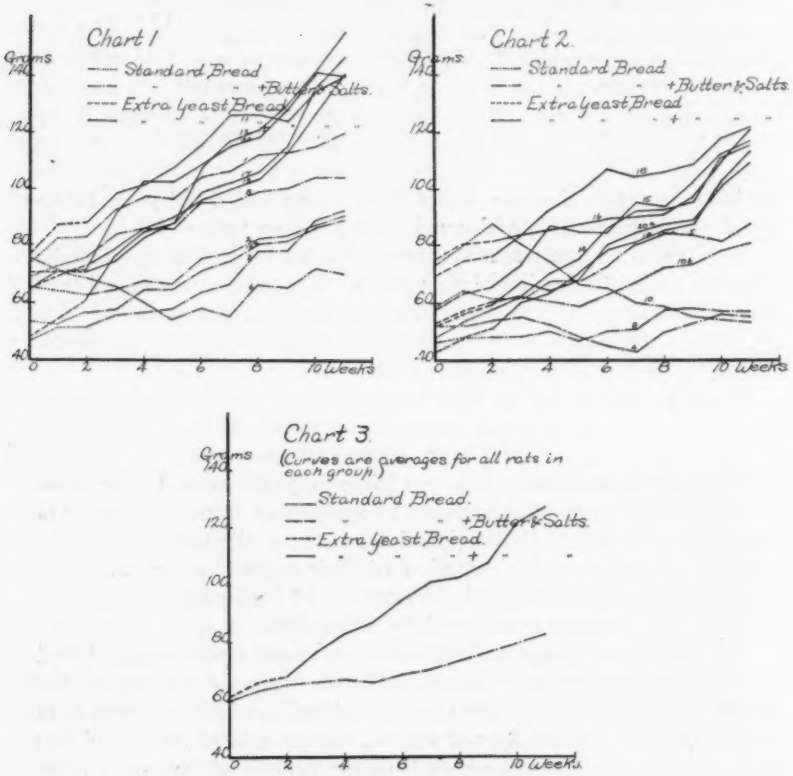
At the end of two weeks, butter and a mixture of inorganic salts (8) were added to the diet of each group. The rats were weighed each week. The weights of the individual rats are shown in charts 1 and 2. In chart 3 the results are summarized by means of average curves for the rats on standard and on extra yeast bread.

It will be noted that on the average the rats fed extra yeast bread gained 66 grams in 11 weeks or nearly three times as much as the rats fed standard bread (23 grams), average gains per week being 6 grams and 2 grams respectively. The rats fed extra yeast were also far superior in general physical appearance as can be seen from the pictures of rats 4 and 14 (fig. 4) that weighed the same at the beginning of the experiment and were fairly typical of their respective groups. Rats 3 and 13 (fig. 5) also weighed the same at the beginning of the study, and their pictures are reproduced for comparison.

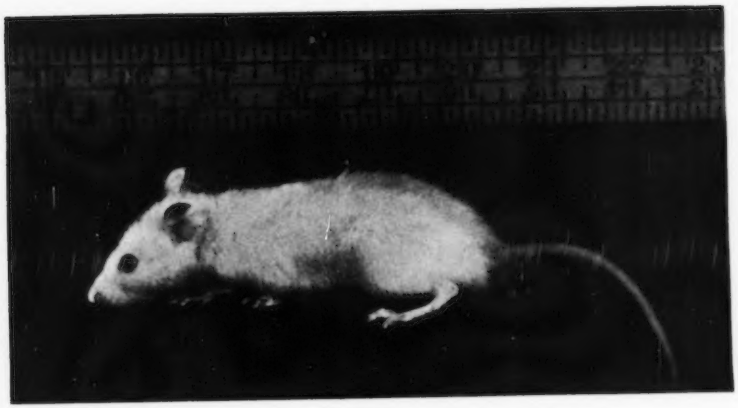
The bread used as a standard was by no means a poor quality bread but on the contrary was somewhat better than the average as the liquid used in its preparation was one-half milk, which is absent from some products. About 10 per cent of the protein of this bread was milk protein, while about one-fifth of the protein of the extra yeast bread was yeast protein, the latter also of course containing milk, to make a total of about 30 per cent of non-wheat protein.

Patent wheat flours and breads made from them without suitable addition are known to be deficient in water-soluble vitamine and in protein (9). The use of milk to the extent of one-half the liquid required or sufficient to replace 10 per cent of the wheat protein by milk protein did not lead to significant gains in weight, in spite of the higher quality of the proteins of milk. That the milk addition should

furnish sufficient water-soluble vitamine would not be expected from the relatively low vitamine content of milk as indicated by the work of Osborne and Mendel (10), who found even 15 cc. of fresh unpasteurized summer milk inferior to 0.2 gram of dried yeast in this respect.



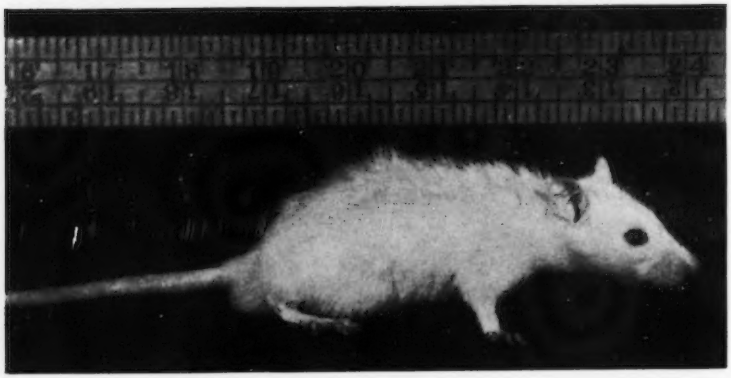
The addition of 5 per cent of dried yeast to the flour, therefore, undoubtedly increased the content of water-soluble vitamine from a very low to an adequate level and also supplemented the protein of the flour in an important way. The fact that even with this 5 per cent yeast addition maximal growth was not attained indicates that a greater amount of yeast could be used to advantage in further improving the bread protein as well as for the stimulatory action of the yeast on



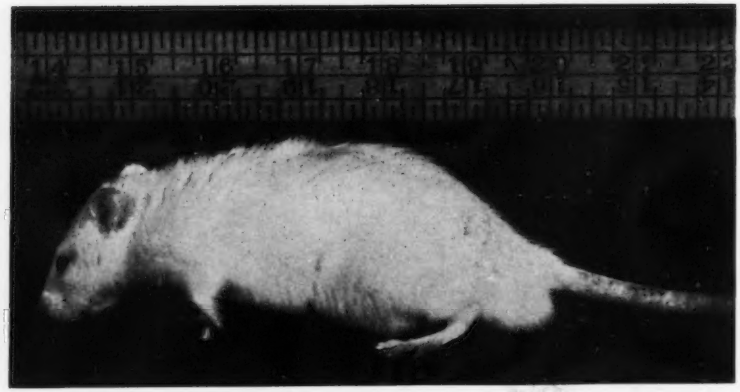
Rat 4

Rat 14

Fig. 4



Rat 3



Rat 13

Fig. 5

protein metabolism, which may be associated with the high vitamine content of yeast. Because of this high content of yeast in water-soluble B less of it would be required to make the bread adequate in this respect than to supplement properly its protein, and smaller amounts than would be necessary for the latter purpose would be of value in connection with diets containing moderate amounts of meat and milk, which would adequately supplement the bread protein, but which are low in water-soluble vitamine.

CONCLUSIONS

Flour containing 5 per cent of yeast powder makes a palatable bread much more nutritious than ordinary bread. The yeast supplements both the water-soluble B and protein content of wheat flour. Yeast is thus a nutrient constituent of bread, and any increase in its amount up to quantities far in excess of those ordinarily used will improve the food value of the product.

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THE EFFECT OF SUCKLING AND CASTRATION ON THE LACTATING MAMMARY GLAND IN RAT AND GUINEA PIG

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According to Jentzen and Beuttner, Thomas Winn, an American farmer, first made the observation that in castrated cows milk production may continue for several years (1). Since then this observation has been frequently confirmed and the effect of castration on the composition of the milk studied. Marshall and Jolly moreover found that castration carried out during the latter part of pregnancy permits further growth of the mammary gland and subsequent lactation (2).

It has furthermore been observed that cows which are not milked "run dry," that evidently suckling acts as a stimulus of secretion (3). To our knowledge a systematic study of the course of lactation and the influence of suckling and castration on the mammary gland following labor has not yet been made. We used for our experiments the same rats and guinea pigs which served for the two preceding studies which concerned the uterus and ovaries in the period following labor.

THE MAMMARY GLAND IN NURSING RATS AND GUINEA PIGS

Six to twelve hours after labor the mammary glands in rat and guinea pig are in a similar condition. In both as a result of the proliferative processes during pregnancy the glands are large, but the epithelial cells have not yet reached their full size, neither are the acini as yet usually distended, although we find some acini with a larger lumen in the rat. A limited number of vacuoles indicate in both the onset of secretion.

There are, however, some differences between the guinea pig and rat. In accordance with the observations of Loeb and Hesselberg (4) we find in the guinea pig soon after labor a number of mitoses in acinus cells,

while they are quite rare in the rat. This corresponds to the effect of heat and ovulation on proliferative processes in the mammary gland of the guinea pig, which we described previously. Accordingly we find also a few mitoses in the duct epithelium of the guinea pig, but not of the rat. The condition of the stroma corresponds to the state of activity in the parenchyma. Thus we find in the guinea pig the connective tissue around the acini cellular consisting of fairly large fibroblasts with vesicular nuclei which show some mitoses, while in the rat the condition of the stroma is intermediate, the stroma consisting of thin strands of fibrous or fibrillar tissue with small cells and with a much smaller number of mitoses. In the rat, on the other hand, this growth period which in the guinea pig we called primary is absent.

Two days after labor we find in both rat and guinea pig further progress toward active secretion, but in the guinea pig the stage reached is somewhat further advanced than in the rat. In the rat the gland is red in color due to the presence of many distended capillaries, in the guinea pig the presence of milk gives it a reddish-white appearance. In both the gland epithelium has enlarged since labor, but in the guinea pig it has reached about the maximum size, while in the rat this stage has not quite been reached.

In both rat and guinea pig many gland cells contain vacuoles, indicating active secretion; but in the rat we find now in addition an active stage of mitotic proliferation in the gland cells which has apparently already passed in the guinea pig. In the rat we find accordingly also some mitoses in the gland ducts while they are absent in the guinea pig. In accordance with the activity in the parenchyma at this stage there is in the rat also more proliferation in the connective tissue cells than in the guinea pig. This proliferation is probably in some way connected with those conditions which lead to secretion. It is due to a stimulus which developed in the transition to the state of secretion in the gland cells rather than to the action of an ovarian hormone.

In both the stroma consists of thin fibers with small connective tissue cells. There is no marked difference between the castrated and non-castrated animals, but in the rat the number of mitoses is a little larger in the non-castrated animals, while the gland cells are of greater size in the castrated rats. In the stroma of the non-castrated guinea pig the number of mitoses is greater than in the castrated animal; these in all probability represent merely accidental variations.

There is, however, one noticeable difference between the rat and the guinea pig which remains constant throughout the period of lactation.

In the former amitotic proliferation of the nuclei is rare at all times, while in the guinea pig amitotically dividing nuclei are frequent. Amitoses and mitoses are to some extent mutually exclusive. We find therefore in the rat the number of amitoses as yet smaller than later in accordance with the fact that an active mitotic proliferation is going on at this period. Marked mitotic proliferation is furthermore incompatible with active secretion. Accordingly secretion has not yet reached full development in the rat at this period.

This proliferation in the mammary gland of the rat does not depend upon a substance given off by either corpus luteum or ovary as a whole, because it is present also in castrated animals, although not to so marked an extent as in non-castrated animals. The early proliferation in the guinea pig evidently depends upon the same factor that determines heat and ovulation.

In the guinea pig we observe in this specimen as also in later specimens a coalescence of some adjoining acini, the separating stroma being dissolved.

Seven days after labor secretion is fully established in the rat. Many acini contain large lumina filled with secretion. While the epithelium of the glands has reached its full height, in some of the larger acini the epithelium is flat probably as the result of the pressure of the increased secretion. Here also adjoining acini may coalesce. In accordance with the full establishment of secretion mitotic proliferation has now ceased and amitotic proliferation of the nuclei is somewhat more frequent, but in the rat amitoses remain on the whole rare. As in the guinea pig so also in the rat the higher epithelium of the glands,—and this applies especially to those cells in which the nuclei divide amitotically—the cytoplasm, takes on a bluish hemotoxylin stain. Around the acini the connective tissue forms a thin fibrous coat with small connective tissue cells which show very rare mitoses. There is no essential difference between the normal and castrated animal.

In the guinea pig conditions are similar to those found two days after labor. Secretion is fully established. Neighboring acini may fuse together. Some very dilated acini have flat epithelium as the effect of pressure. A few mitoses appear in the acini of the castrated guinea pig, and they are still somewhat more frequent in the ducts of the castrated animal. Mitoses happen thus in this case to be more frequent in the castrated than in the non-castrated guinea pig.

Two, three and four weeks after labor conditions are very similar in the mammary glands of rats to those found after one week. There exist

however some slight differences. In all of them fusion of neighboring acini may occasionally occur and this may lead to the formation of small cystlike formations.

While the gland epithelium is usually high in all of these animals, some more dilated acini have flat epithelium, the vacuolar part of the cytoplasm having already been discharged. At two and four weeks only exceptional mitoses are found, both in the castrated and non-castrated animals, and in parenchyma as well as in stroma. At three weeks mitoses are more frequent in the parenchyma (gland and duct cells) as well as in the stroma of the castrated animal. This mitotic proliferation must therefore be caused by conditions in the gland itself or its direct environment; it cannot be due to an ovarian hormone. On the whole the glands in the castrated and non-castrated animals behave exactly alike. There are always a few amitoses present in both. After three and four weeks a certain increase in the amount of red staining material is noticeable in gland and duct lumina. This may either indicate that the suckling has become less active at this time and that consequently a slight stagnation in the secreted material has occurred, or it may indicate that secretion is proceeding very actively, that consequently more material is produced than can be removed through suckling.

In the guinea pig conditions after two weeks are similar to those found after seven days. Occasionally neighboring acini fuse; some acini are more distended and lined with low cuboidal or flat epithelium. Mitoses are absent, but frequent amitoses are found. There is no difference between the non-castrated and the castrated animal. After three weeks some slight changes are noticed. At this period we are at or near the point where the transition from intense activity to a marked diminution in the nursing occurs. We find more colloid-like eosin staining material in gland lumina and ducts. Nuclear amitoses in gland cells are most frequent at this period and at some parts the epithelial cells are degenerating through vacuolization or pyknosis. We find a considerable hyperemia in both glands; some lymphocytes and polynuclear leucocytes are scattered in the stroma and occasionally small masses of leucocytes are found around and inside of some acini. These facts evidently indicate the presence of disturbing factors, perhaps due to a disproportion between secretion and removal of secreted material. It initiates the retrogressive changes. Exceptional mitoses are found in the parenchyma and stroma in the non-castrated animal. In all other respects the glands are like those found at two weeks. There is no difference between the castrated and the non-castrated animals.

The beginning of the retrogressive changes in the mammary gland of the guinea pig is much more marked after four weeks. At this period the guinea pigs no longer nurse their young ones to the same extent as before and the mammary gland is therefore smaller in both animals. The gland is still composed of many lobules, but they are much smaller than formerly and each lobule consists of many acini which are likewise now much smaller, although there are still some with a medium sized or large lumen. The gland cells also are smaller and lower; they are medium or low cuboidal. The number of vacuoles in the gland cells has much decreased and those present are small. The number of amitoses is likewise much smaller, but in some of the larger acini a few amitoses are still observed. The number of mitoses on the contrary has increased; we find about two or three mitoses in each section in the gland cells and also a certain number of them in the stroma, especially of the castrated animal. As usual a certain reciprocal relation exists between amitotic and mitotic activity, and again we find a certain parallelism between mitoses in parenchyma and stroma.

A direct destruction of gland tissue is indicated by the presence of karyolysis and karyorrhexis in some acinus cells. In accordance with all these findings the quantity of colloid-like material in the glands and ducts has much decreased.

While formerly only a thin fibrous coat surrounded the acini, we now find a large amount of dense fibrous tissue around the acini. Again some lymphocytes and polynuclear leucocytes are scattered in the stroma; some of the lymphocytes even are migrating through the epithelium of the acini and ducts and we find some masses of polynuclear leucocytes around and in some acini. This transitional stage evidently leads to disturbances which attract lymphocytes and polynuclear leucocytes. As we see, conditions are essentially similar in the castrated and the non-castrated animals.

After five weeks we find for the first time decided retrogression in the rat, while in the guinea pig, in which it had begun somewhat earlier, retrogression has made further progress at this time. In the rat the mammary gland has become much smaller; the lobules are smaller and each lobule consists of a much reduced number of acini which have a smaller lumen than formerly. The individual gland cells likewise are smaller. The number of vacuoles in the gland cells is reduced and there is less colloid-like material in the lumen of the acini and ducts. The connective tissue around the acini has now generally become densely fibrous and it is partly edematous. Those lobules which are surrounded by

dense connective tissue, usually consist of especially small acini; this may be due to some pressure exerted by the stroma. Some lymphocytes and polynuclear leucocytes are scattered in the stroma and some of them migrate through the glandular epithelium. In the castrated animal the mammary gland is similar.

We find therefore at five weeks in the rat conditions corresponding to those seen in the guinea pig after four weeks. There is a further similarity. Just as we found in the guinea pig with beginning retrogression at four weeks a decrease in amitoses and in other signs of secretion coinciding with a relative increase in mitoses, so we find also in the rat some increase in mitoses at five weeks; about two to three amitoses can be seen in the gland cells in each section. A few mitoses are also found in the ducts and in the stroma; again these mitoses are due to local changes and not to influence of an ovarian hormone.

In the guinea pig retrogression has still further progressed at five weeks. In both the castrated and the non-castrated animals the mammary gland has further decreased in size; the gland is still composed of many lobules, but they are now much smaller and each lobule consists of a smaller number of acini which latter are also generally very small. Some acini, however, are still somewhat larger. The epithelial cells are now lower and the number of vacuoles and also of amitoses has decreased considerably. They are observed in those acini which are still relatively larger. In the non-castrated animal we again find a relative increase in mitoses in the parenchyma as well as in the stroma, while in the castrated guinea pig mitotic proliferation has ceased at this time. The colloid-like material in acini and ducts is small in amount; the stroma is densely fibrous. In the non-castrated guinea pig the nuclei of the acinus cells are on the whole pale, while they take the stain well in the castrated animal. In both we find epithelial cells which degenerate by vacuolization, karyolysis or karyorrhexis; the degeneration is somewhat less pronounced in the castrated animal. In both guinea pigs many lymphocytes and some polynuclear leucocytes are found in the stroma and some of them migrate through the epithelium of acini and ducts, and collecting around and inside of acini, they may contribute to the destruction of the gland.

THE MAMMARY GLAND IN NON-NURSING RATS AND GUINEA PIGS

Two days after labor. In both rat and guinea pig the effect of the lack of nursing is noticeable as early as two days after labor.

In both the nursing and the non-nursing rat at this period the mammary glands are large, but while in the nursing animal they are red colored, indicating an active circulation, in the non-nursing rat they are pale. The lobules are somewhat smaller in size in the non-nursing rat. In the non-nursing animal the lobules consist of large acini, but this is mainly due to the considerable amount of vacuolar and colloid material found in the lumen of the acini and in the ducts. The ducts are dilated by the stagnating secretion. The gland cells themselves are here distinctly lower than in the nursing rat; they are low cuboidal or flat, although some cells are a little higher. Again we notice a coalescence of some neighboring acini. As in the nursing rats the gland cells contain vacuoles, which are more frequent in the non-castrated than in the castrated animals, but the nuclei are on the whole pale in the non-castrated rat, while in the castrated rat the nuclei stain well with hematoxylin. While in the nursing rats rare amitoses are found, they are lacking altogether in the non-nursing rats, indicating a cessation or diminution in secretory activity in the latter animals. On the other hand, a certain number of mitoses are observed in the gland cells of the non-castrated rat, they were exceptional in the castrated animal. In the nursing animals the stimulus of nursing evidently called forth a much greater mitotic proliferation. The character of the stroma is not yet changed, but the number of mitoses in the stroma is reduced in the non-nursing rats. There are as yet very few lymphocytes and polynuclear leucocytes in the stroma. With exception of the few differences mentioned the glands in the castrated and the non-castrated rats were similar.

On the whole we find analogous conditions in the nursing guinea pigs after two days. The glands differ here from those of the nursing guinea pigs in the following points: *a*, The gland cells are not quite so high; *b*, in the non-castrated animal the nuclei of the gland cells are pale; they stain well in the castrated animal; *c*, while in the nursing animals small vacuoles were seen at this period, in the non-nursing guinea pigs many epithelial cells contain granules. Some granules get into the lumen of the acini and some acini are filled with granules. *d*, in the non-nursing guinea pig some gland cells are degenerating (vacuolization, pyknosis, karyolysis, karyorrhexis); *e*, amitoses are frequent in the nursing, and very rare in the non-nursing animals; *f*, colloid-like material is more plentiful in the lumen of acini and ducts in the nursing than in the non-nursing animals; *g*, mitotic proliferation is rare in the parenchyma of the non-nursing animals, but a few mitoses are found while they are absent in the nursing guinea pig; *h*, in the non-nursing, non-cas-

trated guinea pig there is already a small increase in lymphocytes and polynuclear leucocytes in the stroma. This increase is lacking in the castrated animal.

Seven days after labor a great decrease in the size of the mammary gland has taken place in the rat, the diminution between the third and seventh day following labor is very marked. The gland is now of small size, while at two days it was still large; both the number of lobules and of acini is much diminished. There are only few lobules and few acini left in both the castrated and the non-castrated rats. The acini are of small size, they have a small lumen and low cuboidal epithelium which is also of small size. The absorption of the secreted material has much further progressed in the non-castrated animal; here very little colloid is visible in acini and ducts and all the ducts have a narrow lumen; there is more colloid-like material left in the acini and ducts in the castrated rat; here some of the ducts are even cystically dilated by retained secretion. Thus it comes about that in the castrated rat some acini appear larger than in the non-castrated rat. In some gland cells blood pigment can be seen. Only very few of the epithelial cells contain vacuoles. Evidently the back pressure of the secretion and the lack of the stimulating effect of the suckling cause a cessation in the formation and secretion of milk. As in all non-nursing rats amitoses are completely absent. On the other hand, in contradistinction to the nursing rats, there is still some mitotic proliferation going on in the gland cells of the non-nursing, non-castrated animal; it is absent in the castrated animal. The connective tissue has relatively increased around the acini; it is of a fibrous character, mitoses in the stroma are very rare.

In both the castrated and the non-castrated rats there are many lymphocytes and some polynuclear leucocytes present in the stroma; occasionally the polynuclear leucocytes form small collections. Some of these cells migrate through the glandular epithelium and thus they may injure some of the acini and ducts.

In the non-nursing guinea pigs the same process occurs at this period, but the retrogression is not so rapid as in the rat. As in the non-nursing rat the mammary gland is smaller than that of the non-nursing guinea pig two days after labor; it comprises still many, but much smaller lobules and each lobule has less acini which have a small or medium sized lumen. In the non-castrated, but not in the castrated guinea pig, there are occasionally some large acini present. The epithelium is low, cuboidal or flat in the acini of medium or small size, and somewhat higher and larger in some of the larger acini. It is therefore at places higher

in the non-castrated than in the castrated guinea pig. Some of the higher cells have large vacuoles and the nuclei divide here amitotically. There is therefore more vacuolization and more amitotic division in the non-castrated animal. There are only very few amitoses in the castrated guinea pig; but they are also much diminished in the non-nursing, non-castrated guinea pig as compared with the nursing, non-castrated guinea pig at the corresponding period.

The more active secretion in the non-castrated animal is also indicated by the greater amount of colloid-like material in the larger acini as well as ducts of this animal. The stroma is relatively more fibrous than at two days in the non-nursing animals and also as compared to the nursing guinea pigs; it is especially dense around the smaller acini. The connective tissue nuclei are small.

In contradistinction to the amitoses, we find a considerable number of mitoses mainly in the gland cells, less in the gland ducts. The mitoses in the connective tissue cells are likewise much increased. In the nursing guinea pigs mitoses are lacking or very rare at this time. In a similar way we find an increase in the number of mitoses in the non-nursing rat at this period, but in the rat it is still more marked in the following period.

In the non-castrated guinea pig many lymphocytes and some polynuclear leucocytes are found in the stroma and some of them migrate through the epithelium of the gland and destroy some parts of it. In the castrated animals the number of lymphocytes is much smaller; again they injure here some of the acini.

In contradistinction to the rat we again find in the guinea pig marked degenerative processes in the gland tissue. Vacuolization, karyolysis, pyknosis and karyorrhexis are seen in the gland cells. Lymphocytes may participate in this destruction. At some places the epithelium has disappeared, and the colloid material is entering the stroma, and connective tissue begins to proliferate and to fill spaces formerly occupied by gland tissue. In the castrated guinea pig the amount of degeneration is less.

Two weeks (15 days) after labor. In the rat the size of the gland has still further decreased and has almost reached its lowest level. It consists of very few and small lobules and isolated ducts. Each lobule comprises very few small acini. The gland cells are small and only a few of them contain vacuoles. Little colloid-like material is found in acini and in the ducts, which latter have a very narrow lumen. The stroma is fibrous, but small in amount; it contains some lymphocytes

and polynuclear leucocytes, some of which migrate through the epithelium of acini and ducts. There is a considerable mitotic proliferation going on in the glands of the castrated and the non-castrated animals; it is more marked in the latter. The mitoses in the stroma are exceptional. In the castrated rat the lumina of the acini are furthermore smaller and they contain less secreted material than in the non-castrated animal. There are also fewer vacuoles present in the castrated rat. Otherwise both behave alike.

In the guinea pig a more steady, less abrupt decline in the size and activity of the mammary gland takes place; the gland is small and contains fewer lobules and fewer acini than formerly. In general the acini are small and the gland cells are low cuboidal and also of small size; however, around the larger ducts there are some acini with a large lumen and lined by high cuboidal epithelial cells. It is especially these higher cells which contain vacuoles and divide amitotically or degenerate in the usual manner. On the whole the occurrence of vacuoles and amitoses in the gland cells is infrequent. Some products of secretion are seen in the large acini and in the ducts. The stroma is fibrous; there is more dense fibrous tissue around the small than around the larger acini.

There are many lymphocytes and some polynuclear lymphocytes in the stroma and some of them are passing through the epithelium and destroying it. Mitoses are quite frequent; but they are more frequent in the non-castrated than in the castrated animals; in the former they are also frequent in the stroma. In the castrated guinea pig not only the mitoses are less, but the high gland cells are missing; there are furthermore fewer amitoses, fewer degenerating cells and a smaller number of lymphocytes and polynuclear leucocytes.

After three weeks the mammary gland in the non-nursing rat is similar to that found after two weeks. Glands and acinus cells are small. At this time as after two weeks some blood pigment can be found in the epithelial cells. The ducts are mostly isolated, unaccompanied by acini. Some masses of lymphocytes and polynuclear leucocytes are encountered around some acini and some of the lymphocytes migrate through the glandular epithelium.

The number of mitoses in the gland tissue proper happens to be much reduced in this specimen; they are lacking altogether in the stroma. The gland of the castrated animal differs from that of the non-castrated rat in the following points: In the former the acini and the gland cells are somewhat smaller; there are less vacuoles, less mitoses, less products of secretion; furthermore the number of lymphocytes and polynuclears is smaller.

In the guinea pig also the glands are on the whole similar to those found after two days and we notice the same differences between the glands of the non-castrated and the castrated animals as before. However, large acini are now absent and degenerating cells are no longer found; amitoses are still rarer than before. Mitoses are not infrequent; they occur in both parenchyma and stroma. While quite a number occur in the gland cells in the castrated guinea pig they are here not so frequent as in the non-castrated animal. In the castrated guinea pig the mammary gland as a whole is smaller and the individual lobules and acini are also smaller. The acini lined with larger and higher epithelial cells are here not visible. The number of vacuoles, and the quantity of secreted material in the acini and the number of lymphocytes in the stroma is less; but even in the castrated animal we find some masses of lymphocytes and polynuclear leucocytes around and inside some acini.

Four weeks after labor the mammary gland in the non-castrated, non-nursing rat is small, but perhaps not quite so small as at three weeks; the number of lobules and acini is slightly larger. The main difference, however, consists in the very much larger number of mitoses which we find here everywhere in acinus, duct cells and stroma. Some ducts have still a wide lumen. Some acinus cells and connective tissue cells contain blood pigment.

Where there existed in the previous periods some difference between the non-castrated and the castrated non-nursing rats, it is still more marked in these specimens. In the castrated animal the gland is smaller, consists of fewer lobules and acini which latter are very small; the gland cells themselves are also much smaller. The ducts have a narrow lumen and the amount of secreted material is less. The number of mitoses is very much less in all the structures. There are here fewer lymphocytes and polynuclear leucocytes in the stroma than in the non-castrated animal.

In the guinea pig at this time no marked change has taken place from the preceding period except that the number of mitoses has decreased very much in both the non-castrated and the castrated animals; they are very rare in the non-castrated and absent in the castrated animals, The number of vacuoles and amitoses in both animals is very small. There is a very small quantity of thick colloid-like material in some acini and in the ducts. Again there are in the non-castrated animal many lymphocytes and polynuclear leucocytes present and some of them migrate through the gland epithelium; occasionally large collec-

tions of the lymphocytes are observed around and in some acini which are partly destroyed. In the castrated animal the number of lymphocytes and polynuclear lymphocytes present is smaller.

After five weeks the mammary gland in both the castrated and the non-castrated rat is very small and hardly visible to the naked eye. It is composed of very few lobules and isolated ducts. In each lobule there are very few and very small acini which are lined with small low cuboidal epithelium. Mitoses are now exceptional in the glands of both the castrated and the non-castrated rat. Colloid-like material is very small in amount or absent. Very few acinus cells have vacuoles and they are fine. The ducts are very narrow. The stroma around the acini consists of little fibrous connective tissue, with small cells. Some epithelial and stroma cells contain blood pigment. There are some lymphocytes and polynuclear leucocytes in the stroma and some of the lymphocytes are passing through the gland epithelium.

In the castrated animal the acinus cells are somewhat smaller and there are fewer lymphocytes and polynuclear leucocytes present in the stroma; otherwise both glands are similar. At this time the lowest point in both size and activity of the glands has approximately been reached in the glands of the castrated and the non-castrated rats.

In the non-nursing guinea pigs the mammary gland also shows a still further decrease in size from the former period. It consists now of a few lobules and a few small acini; the acinus cells are small and low. Only exceptional mitoses and very rare mitoses are visible. The number of vacuoles and the amount of colloid-like material is very small. The stroma is fibrous and partly slightly edematous. There are many lymphocytes and some polynuclear leucocytes and some of them collect around the glandular epithelium and migrate through it. In the castrated guinea pig the number of lymphocytes is smaller.

We see then that at five weeks in both non-nursing rat and guinea pig the lowest state has been reached and that at this time the difference between the mammary gland of the castrated and the non-castrated animal has almost disappeared.

DISCUSSION

1. Curves of activity and size of the mammary gland in the nursing animals. In both rat and guinea pig the mammary gland has not yet reached its maximum secretory activity ten or twelve hours after labor. This is attained in the guinea pig two days and in the rat about seven

days after labor, or somewhat earlier; the maximum is therefore reached earlier in the guinea pig. From this period on the gland maintains approximately the same condition for several weeks. In the rat a maximum of secretion is found three and four weeks after labor; then a sudden diminution in the size of the glands and of the gland cells is noticeable five weeks after labor. This goes hand in hand with a diminution in those signs which indicate secretory activity in the gland. In the guinea pig a critical point is reached three weeks after labor, when we find associated with certain conditions indicating a maximum of secretion (maximum of amitoses, much colloid material) the first signs of degeneration in some gland cells. In the following two weeks we find involutionary changes, which are much more marked at five than at four weeks after labor. In the guinea pig in which the young at the time of birth are much more mature than in the rat, suckling ceases at an earlier period.

Five weeks after labor we find in the rat a marked decrease in the size of lobules and acini, in the number of acini and in the size of the gland cells; in the guinea pig a similar decrease occurs four and five weeks after labor.

2. Besides changes in the size of the lobules and acini and in the size and shape of cells, we find changes in the processes of secretion, namely, in the number and size of vacuoles, the frequency of amitoses in the acinus cells and the amount of colloid-like material in the lumen of the acini and ducts.

In the rat the number of vacuoles increases in the first two days after labor; a decrease occurs at five weeks. In the guinea pig the initial increase is simultaneous with that in the rat; the decrease in number and size of vacuoles is very marked at four weeks and still more pronounced at five weeks.

Amitoses occur in the rat as well as in the guinea pig when the gland cells have reached a large size; but throughout they are much more frequent in the guinea pig than in the rat. They decrease in rat and guinea pig with the cessation of suckling. The diminution in the colloid-like material at this period is especially noticeable in the guinea pig.

3. The mitotic proliferation in the glands shows an interesting relation to the activity or cessation of activity in the glands. In the rat an active mitotic proliferation in the parenchyma and stroma is found two days after labor; this coincides with the peroid of ascent toward full secretory activity. At that time the number of amitoses in the

rat has not yet reached a maximum. When the secretory activity is fully established mitotic activity is very low; mitoses are exceptional and occasionally a somewhat increased number occurs, as for instance three weeks after labor in the castrated rat. But with the cessation of secretion five weeks after labor, at a time when the number of amitoses is much diminished, the mitotic activity has again become quite active.

In the guinea pig we find a marked mitotic activity soon after labor, at a time when a new ovulation is imminent and the animal is in heat. With the full development of secretion and of amitotic activity mitoses become very rare in parenchyma and stroma; but as in the rat with beginning cessation of secretion and diminution in the number of amitoses the mitoses become again frequent after four weeks; this mitotic activity is still present but with diminished intensity five weeks after labor. On the whole mitoses in the parenchyma and in the stroma follow a parallel course. As a rule an increase in the number of mitoses in the stroma is found concomitantly with an increase in the parenchyma; but occasionally mitoses are increased in the stroma without a simultaneous increase in the parenchyma and vice versa. It is possible that sometimes the increase in the mitotic activity of the stroma has just passed or not yet begun at the time of examination. On the whole this parallelism to which we had drawn attention previously is unmistakable.

This appearance of what might be designated as alternating proliferating activity in rat and guinea pig does not depend on hormones secreted by the gonads; it occurs also in castrated animals. It is in the main due directly to changes in the mammary gland itself.

4. While in the actively proliferating mammary gland of the guinea pig the stroma is at least in parts rich in fibroblasts, in the secreting gland of the nursing animal the stroma is fibrous in the guinea pig as well as in the rat. However, the amount of fibrous tissue around the secreting acini is very small. As soon as the secretion ceases, the amount of fibrous tissue around the acini increases considerably. This is perhaps to a great extent due to a retraction of the fibrous stroma which has been made possible through the diminution in the size and number of the acini.

5. Lymphocytes and polynuclear leucocytes are found primarily in the stroma. Secondarily they often collect around certain acini and penetrate through the acini or lining of the ducts into the lumen of the gland. In the rat as well as in the guinea pig they appear when the secretion begins to cease and an involution of the gland sets in. Thus

we find them in the rat five weeks after labor; in the guinea pig they appear earlier, namely, three weeks after labor; they are visible four weeks after labor and become still more numerous at five weeks. A small number of them is seen in the guinea pig soon after labor and in the beginning of secretion. Somehow the changes produced by cessation of secretion attract these cells. They are on the whole less numerous in the castrated animals. This is especially noticeable in the series of non-nursing animals; but it is likewise evident in the series of nursing animals five weeks after labor.

6. The mammary glands in the castrated and the non-castrated rats and guinea pigs are essentially alike during the period of secretion. The effect exerted by the ovary is negligible in comparison with the effect exerted by the suckling and with the changes brought about through the sudden cessation of growth stimuli in a mammary gland in which hyperplasia had been previously caused through the growth factors active during pregnancy.

There are minor differences between the castrated and the non-castrated animals; they are probably of an accidental character, but our findings in the non-nursing animals where the differences are more definite suggest that also in the first series certain differences between the castrated and the non-castrated animals may be due to the influence of the ovary. They are the following: *a*, Two days after labor the number of mitoses is greater in the mammary gland of the non-castrated than of the castrated rat. In a similar way the number of mitoses is much greater in the mammary gland of the non-castrated than of the castrated guinea pig five weeks after labor. We interpret these facts as indicating that the effect of the ovarian hormone may be effective in the time following secretion or preceding it. *b*, In the rat the number of lymphocytes in the stroma of the mammary gland five weeks after labor is less in the castrated rat than in the non-castrated rat. This difference between the castrated and the non-castrated animals is more pronounced in the series of non-nursing animals.

7. It might be asked whether the growth stimuli which cause the growth of the mammary gland in the normal pregnant guinea pig might be equally efficient in causing proliferation in the gland of the guinea pig, nursing her young in the period following labor. In our previous paper we gave some facts which make it very probable that in the suckling guinea pig these growth stimuli of pregnancy are ineffective (4). We found that fifteen, nineteen and twenty-nine days after labor in guinea pigs which had been impregnated directly following

labor and which suckled their young, mitotic proliferation was absent in the glands, that on the other hand the typical amitotic proliferation of the nuclei was in evidence.

We may then conclude that the processes active during secretion successfully counteract the influence of the growth stimuli of pregnancy.

8. The curves of involution of the mammary gland in the non-nursing animals take a somewhat different course in the rat and in the guinea pig. In the rat the descent is very rapid; it is already noticeable after two days, becomes very marked after seven days, and has almost reached its lowest point at fifteen days following labor; it progresses extremely slowly during the following four weeks. Lobules and acini decrease in number and size; epithelial cells become smaller. In the guinea pig the main decline takes place also in the first two weeks, but the decrease is somewhat less abrupt and proceeds more evenly toward end of the fifth week. The essential changes involved in involution are in both species the same. This difference in the curves is perhaps referable to the fact that in the rat the gland tissue is distributed over a much wider area than in the guinea pig where the gland is concentrated in two places. It is of interest to compare these curves with those of the retrogressing uterus. Here we also find a more rapid decline in the rat than in the guinea pig.

9. The changes found in the non-nursing animals are the same as those found in the nursing animals after cessation of suckling, namely, besides the diminution in size and number of lobules and acini a diminution in the number of vacuoles, in the size of the acinus cells, in the amount of colloid-like material in acini and ducts, in the character of the stroma, which becomes more densely fibrous and is present in a relatively larger quantity in the nursing animals. The number of nuclear amitoses is much diminished in the guinea pig; they are absent in the non-nursing rat.

There is an additional difference between rat and guinea pig; in the latter degenerative processes in the gland cells are much more marked than in the rat. In the guinea pig gland cells degenerate under the appearances of pyknosis, karyorrhexis and karyolysis and cytoplasmic vacuolization in the first two weeks following labor; a maximum in these changes is reached about one week after labor. At that time whole parts of the gland may be substituted by connective tissue. In the rat these degenerative processes are not noticeable. This difference is perhaps again due to the fact that in the guinea pig the whole gland tissue is concentrated in two areas; therefore the destructive processes are here more violent than in the rat.

10. We found in the series of nursing animals a migration of lymphocytes and polynuclear leucocytes from the vessels into the stroma of the gland and from these into the acini and ducts. The same condition we find in a more marked manner in the non-nursing series. Here we find throughout an emigration of lymphocytes and polynuclear leucocytes. It increases in the first few days following labor and is fully developed seven days after labor. In the guinea pig it is at all times much stronger in the non-castrated than in the castrated animals; in the rat the same difference becomes apparent from the third week on.

The substances produced in the gland during the process of involution attract these cells. These processes of involution are therefore more marked in the non-castrated than in the castrated animals.

11. A study of mitotic proliferation in the non-nursing series confirms the conclusions we arrived at in the analysis of the glands of the first series. Here we found that cessation of nursing was accompanied by a resumption of mitotic proliferation in the formerly secreting glands. In the non-nursing rats we note active mitotic proliferation two, seven and fifteen days after labor. At three weeks proliferation is suspended; but at four weeks it is once more active, reaching here a maximum in the non-castrated animal. In the non-nursing guinea pig mitotic proliferation is very active seven days after labor, it is still quite noticeable, although less intense two and three weeks after labor. Four and five weeks after labor it has again become rare. There is in general a correspondence between mitotic activity in the stroma and parenchyma. In the rat we find throughout the non-nursing series and in the guinea pig two and three weeks after labor, a decided inferiority of the castrated animals, as far as mitotic proliferation is concerned. But even in the castrated animals there is present an active endogenous stimulus for cell proliferation which is independent of an ovarian hormone. It is evidently some factor active in the gland itself which at certain times causes this mitotic cell proliferation. This stimulus is probably at least at a certain period identical with the stimulus which calls forth the secretion of milk. At other times it is probably related to those regressive conditions in the parenchyma which follow milk secretion, when we find degenerative processes in the gland or solution of gland tissue. Both of these conditions are of a similar character; in both we have to deal with degenerative processes and with a discarding either of a part of the cytoplasm or of the whole cell. In a certain sense they may therefore be classed among the regenerative stimuli. In the secretory gland these stimuli merely lead to an amitotic division of the nuclei;

but as soon as secretion ceases mitotic proliferation can set in. We find therefore here an alternating relation between amitotic and mitotic proliferation.

12. There is present in addition to the local proliferative stimuli a secondary condition emanating from the ovary which reinforces the action of the local stimuli. Thus we find that in the castrated animals mitotic proliferation is less marked than in the non-castrated animals, although at the time when the endogenous stimuli are at the height of their action proliferation may reach the same intensity in the castrated and non-castrated animals.

13. We have stated that in the castrated animals mitotic proliferation in the mammary gland was less than in the non-castrated rats and guinea pigs. There was furthermore some difference in the amount of infiltration with lymphocytes and polynuclear leucocytes in these two kinds of animals. A comparison of the glands in the non-nursing series shows still further differences between the castrated and the non-castrated animals. In the rat the size of acini, of acinus cells, the number of vacuoles and of the amount of colloid are usually somewhat smaller in the castrated animals. In the guinea pig we find similar differences in the size of the acinus cells and in the number of amitoses in the first three weeks following labor in the non-nursing series.

We may then conclude that the effect of the ovarian hormone on the mammary gland is present even in the period following labor, that its effect is however covered up by the much stronger effect of suckling and that as soon as this stimulus ceases the effect of castration becomes again apparent, although it may be weak.

14. It has been observed that the suckling of the breast on one side may cause the pressing out of milk from the breast of the other side (5). This suggests the participation of reflexes which connect the separate glands in the process of suckling. It was therefore of interest to test the effect of suckling in one breast on the involutionary changes in the other breast, which take place if here suckling has been made impossible through ligating the nipple. This we did in several cases in nursing guinea pigs. In each case we found that the gland which could no longer be used for suckling retrogressed rapidly, in a way comparable to the involution of the mammary gland in the non-nursing guinea pig; on the contrary the gland of the other side which was suckled by the young continued to be large and to produce milk. We may therefore conclude that the process which maintains the active state of lactation in the mammary gland consists solely in the local effect of the withdrawal of the milk.

15. Since Virchow it is customary to distinguish between functional and formative stimuli. The latter lead to multiplication of cells and nuclei, to growth generally and to differentiation, the former initiate those changes which lead to function unaccompanied by growth. The stimuli initiating lactation are of an intermediate character; they produce a combination of processes of secretion and of an abortive growth, usually consisting in amitotic nuclear division, but under certain conditions advancing to mitotic cell proliferation.

In the case of the mammary gland the processes of secretion probably involve a loss of parts of the cytoplasm and it is probably this factor which is responsible for phenomena of growth which we observe during lactation or following it.

SUMMARY

1. Castration does not noticeably modify the condition of the lactating mammary gland in rat and guinea pig. The effects of castration become manifest as soon as suckling ceases.

2. The changes found in the mammary gland during lactation, after cessation of suckling and in animals which have been prevented from suckling are similar in rat and guinea pig, but some differences exist in the time curves in both species in nursing as well as in non-nursing animals; differences exist furthermore in the frequency of amitotic proliferation, and the kind and intensity of degeneration of gland tissue in both species.

3. An alternating relation exists between mitotic cell proliferation and amitotic nuclear proliferation in the mammary gland. The latter is accompanying secretion, the former precedes and follows secretion. Both are associated in a causal way with the cellular processes on which lactation in all probability depends (a partial solution of cytoplasm) or which initiate the return of the gland to the resting condition (total solution of parts of tissue).

4. The stimulus calling forth lactation is intermediate in character between a functional and a formative stimulus and partakes somewhat of the character of both.

5. The stimuli which call forth proliferation in the mammary gland during lactation and directly following lactation are essentially of an endogenous character; there is secondarily added to this stimulus the action of ovarian hormone. Lactation prevents the action of this hormone from becoming manifest. It manifests itself with the cessation of lactation.

6. Secretion not only prevents the ovarian hormone from manifesting itself in the mammary gland, but also those formative stimuli which are active during pregnancy.

7. Involution of the mammary gland in the non-suckling animal is essentially a local process; it is induced in the mammary gland by the lack of use in one particular gland, without reference to the function of other glands.

8. Invasion of the mammary gland by polynuclear leucocytes and lymphocytes occurs especially during the period of involution in contradistinction to that of lactation. It is less marked in the castrated animals.

9. The stroma of the secretory gland consists of thin connective tissue fibers, in contrast with the proliferating gland where the stroma is often cellular. In the resting gland the stroma is densely fibrous.

10. If we compare the influence of castration and nursing on involution in uterus and mammary gland, we notice in the mammary gland a dominance of the effect of nursing over that of castration, while in the uterus the opposite relation exists.

In both species our results show a very rapid effect of castration and nursing on the activities and size in both organs and especially do they indicate a very early diminution in proliferative activities of the tissues as the result of castration.

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DIET AND SEX AS FACTORS IN THE CREATINURIA OF MAN

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The cause of the excretion of creatine by women is as yet unexplained. Krause (1) observed creatinuria regularly immediately following menstruation and considered that the excretion was related to changes associated with the sexual cycle in woman. M. S. Rose (2) and W. C. Rose (3) and collaborators, however, were not able to confirm the work of Krause. They found the creatinuria irregular and without any relation to the period of menstruation. Great variations between individual subjects were also observed. Krause (4) has suggested that women occupy a position intermediate between men and children as far as concerns their creatine metabolism. Men are able to completely metabolize the creatine normally produced in metabolism; in children, the power of conversion or of destruction of creatine is very slight; while according to the conception of Krause, women are able to metabolize the greater part, but frequently not all, of the creatine which is normally the product of the metabolic activities of the muscles. This implies that there is a diminished power of destruction of creatine in women, but as far as is known to the writers, this is based on indirect evidence and has never been demonstrated experimentally. Denis and Minot (5), (6) have recently maintained that the excretion of creatine by women is associated with a high level of protein metabolism, since even in normal women diets high in protein caused the excretion of large amounts of creatine. They had previously shown that in adults of both sexes in cases of Graves' disease (7) and in normal children (8), the output of creatine varied with the level of protein metabolism. With normal men however creatinuria could not be induced by ingestion of excessive amounts of protein. The theory of Miss Denis points to an exogenous origin of creatine from protein and to a definite saturation point of the organism for creatine, beyond which excretion of creatine whether exogenous or endogenous takes place. The muscles of women and children even allowing for individual

variations, would be assumed to have a lower saturation point than those of men since creatinuria may be so readily induced by increases in protein content of the diet in the former. Several investigators (2), (3) have recently reported that they have been unable to confirm the work of Denis and Minot. Denis and Minot (6) however maintain that the diets employed by W. C. Rose (3) were not sufficiently high in protein content to cause creatinuria. It is to be noted that in the earlier experiments of Denis (5), creatinuria was obtained at essentially the same level of nitrogen excretion as was reached in the experiments of Rose. The criticism is also made that the periods of high protein diet followed a low protein diet and were too brief to give opportunity for the production of creatinuria. In three of the four high protein periods with normal women reported by Miss Denis (5), (6), however, creatine appears on the first day of the high protein period despite a previous diet of low protein content.

The experiments of the present series were undertaken to afford additional evidence on the following points since the experimental data of previous workers seemed conflicting. *a*, The relation of creatinuria to the sexual cycle in the normal adult female. *b*, The influence of the protein content of the diet on the creatinuria of women. *c*, The ability of the female organism to destroy creatine administered per os. Is the destruction or transformation of ingested creatine by the adult female influenced by variations in the sexual functions?

METHODS

Creatinine was determined by the microchemical method of Folin, creatine by the method of S. R. Benedict, pure creatinine being used as the standard in both cases, and total nitrogen by the method of Kjeldahl-Gunning.

Three healthy women, students or instructors in the laboratory, served as subjects of the experiments. They were all maintained on a creatine-creatinine free diet during the periods of study. Milk and foods in which milk was present to any great extent were also excluded from the diet. The low protein diet consisted chiefly of potatoes supplemented by bread, butter, fruit and green vegetables. The high protein diets of the earlier experiments consisted mainly of eggs, beans and bread, while later the protein was supplied by glidine, a commercial diabetic food of high nitrogen content, supplemented by legumes and cheese. The food was not weighed, but the intake was

relatively constant in the periods of low protein intake. In the periods of high protein ingestion, however, the consumption of food was not uniform, an attempt being made to secure a maximum

TABLE 1

DATE	SUBJECT S., WEIGHT 56 KGM.			SUBJECT A., WEIGHT 57 KGM.		
	Creatinine	Creatine as creatinine	Total N	Creatinine	Creatine as Creatinine	Total N
1919	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
May 7	1.07	0.0	4.62	1.08	0.0	4.90
8	0.84	0.18	6.72	0.75†	0.0	3.20
9	1.10	0.0	6.00	1.04	0.0	6.70
10	1.18*	0.0	5.81	1.04‡	0.14	5.26
11	0.84*	0.0	4.18	0.84‡	0.10	3.56
12	1.16*	0.0	7.25	1.31‡	0.0	6.82
13	1.04*	0.0	5.50			
14	0.75	0.32	5.74			
15	1.10	0.0	6.50	0.94‡	0.27	6.26
16	0.98	0.16	7.58	0.90‡	0.0	6.97
17	1.08	0.10	5.94	1.04‡	0.0	5.10
18	1.08	0.0	5.09	1.08‡	0.05	5.97
19	0.97	0.04	6.31	1.04	0.17	6.85
20	1.02	0.0	5.60	1.04	0.0	5.90
21	1.10	0.0	5.70	1.04	0.0	5.60
22	0.80	0.0	5.80	1.04	0.3	5.85
23	1.03	0.0	7.22	1.16	0.0	5.18
24	1.03	0.09	9.59	1.16	0.0	6.82
25	1.02	0.06	9.66	1.02*	0.0	4.77
26	1.02	0.13	12.27	1.13*	0.0	4.05
27	1.12	0.0	13.46	1.13*	0.0	6.77
28	0.98	0.0	13.82	1.02*	0.06	5.70
29	1.12	0.0	13.85	1.10	0.0	6.40
30	1.12	0.0	12.90	1.02	0.11	7.15
31				0.98	0.04	7.11
June 1				1.02	0.05	8.63
2				1.07	0.05	6.60
3				1.14	0.07	10.56
4				1.08	0.0	7.37

* Menstrual period.
† Incomplete collection.
‡ Illness, overstrain.

rather than a uniform excretion of nitrogen. The experiments recorded in table 1 were carried out at the close of an unusually difficult year of work incident to the confused conditions of the year

1918–19. A comparison of the results of these experiments with those of later ones in which the same individuals served as subjects shows rather striking differences in the frequency of the occurrence of creatine in the urine. In the earlier experiments warm weather made the continuance of the high protein diets difficult, so that later experiments were more successful from this point of view.

DISCUSSION OF RESULTS

Relation of the excretion of creatine to the menstrual period. According to Krause (1), creatine normally disappears from the urine at the onset of menstruation, reappearing immediately after menstruation has ceased. His experiments are open to the objection that the analyses were made at irregular intervals sometimes a month apart. The results given in the tables of the present study afford data from three women under carefully controlled conditions and include eight complete menstrual periods with fore and after periods. In table 2 are presented data covering two successive menstrual periods with the whole of the intervening period. For one subject (S) the data include five complete menstrual periods.

The findings may be briefly summarized as follows. In four cases creatine was present just before the onset of the menses; in four (leaving out of consideration those days in which creatine was ingested), it occurred during the menstrual period; and in three, creatinuria was observed shortly after menstruation. In no case was a tendency toward creatinuria more marked during or near the period of menstruation than at any other time. In fact except in the first two experiments (table 1), which will be discussed later, creatinuria was not only rare and of very brief duration when it occurred, but the amounts excreted were very small. The results seem to justify the conclusion that the periodic variations in the sexual function in women can bear no direct relation to the intermittent creatinuria frequently observed, a conclusion in harmony with more recent work (2), (3). In this connection it is of interest to recall that although according to Hunter and Campbell (9) the total creatine content of the blood of women is higher than that of men, no variation in the content of the creatine of blood during menstruation has been observed by Wang and Dentler (10). The analyses presented however include only one menstrual and one intermenstrual sample for each individual. The value of their results would be greatly increased if analyses had been made at more

TABLE 2

DATE	SUBJECT S., WEIGHT 56 KGM.		
	Creatinine	Creatine as creatinine	Total N
1919	*grams*	*grams*	*grams*
June 26	1.03	0.00	5.89
27	1.04	0.00	5.18
28	0.24†	0.00	
29*	0.49†	0.00	
30*	0.90	0.04	4.55
July 1*	0.90	0.00	4.94
2*	**1.08‡**	**0.55**	**6.43**
3*	1.08	0.06	6.33
4	1.10	0.00	6.21
5	1.12	0.00	6.71
6	0.94	0.00	5.90
7	**0.99§**	**0.00**	**5.23**
8	1.05	0.00	5.20
9	1.07	0.00	5.10
10	0.95	0.00	4.40
11	0.97	0.00	5.10
12	1.07	0.00	5.66
13	0.89	0.00	5.43
14	1.07	0.00	5.33
15	1.07	0.00	4.83
16	1.05	0.00	6.00
17	1.03	0.00	5.45
18	1.07	0.00	5.20
19	1.11	0.00	5.18
20	0.92	0.00	4.52
21	1.03	0.00	5.08
22	**1.39****	**0.00**	**4.92**
23*	1.07	0.12	5.66
24*	0.92	0.00	5.02
25*	**1.07††**	**0.00**	**4.78**
26*	1.01	0.00	5.15
27*	1.01	0.09	5.88
28*	1.04	0.00	5.44
29	1.05	0.00	4.90
30	1.04	0.00	5.27

* Menstrual period.
† Incomplete collection.
‡ 0.494 gram creatine per os.
§ 0.502 gram creatine per os.
** 0.498 gram creatinine per os.
†† 0.500 gram creatine per os.

frequent intervals on the same individual and checked by urinary creatine determinations, in view of the relation observed by Hunter and Campbell between the creatine content of blood and urine.

Protein ingestion and creatinuria. The failure of other investigators (2), (3) to produce creatinuria in normal women by high protein diets as had Denis (5), (6) suggested that there might be a relation between the menstrual cycle and creatinuria following protein ingestion. The organism might at some period of the menstrual cycle be more easily stimulated to creatinuria by ingestion of excessive quantities of protein than at others. Denis does not indicate in her experiments the relation of the protein periods to the period of menstruation. Accordingly in the present study of the influence of high protein diets an attempt was made to determine whether there was any difference in the influence of the protein at different periods of the menstrual cycle.

In the case of subject S (table 1), the ingestion of larger amounts of protein seemed at first to give rise to creatinuria, but even with continued increase in the urinary nitrogen, creatine disappeared entirely from the urine, suggesting that the metabolic processes may be able to adjust themselves to the higher protein level. With subject A, although the increase in the nitrogen output was not as marked, creatine appeared persistently. This may possibly be attributed to the fact that the subject was suffering at the time from a mild attack of hyperthyroidism (7). In later experiments with the same subject when the thyroid condition was improved (table 4) no creatine excretion was observed to accompany a higher protein level. With subject S at a later period (table 3) creatine appeared only once although the nitrogen excretion was three times as great in the high protein period as in the preceding low protein one. The results indicate that in these subjects, at least, there is no more tendency to creatinuria during menstruation when the diet is high in its content of protein than when it is low; in fact in table 3 during the high protein period there was a total absence of creatine in the urine during the menses. Creatinuria was no more easily provoked by a high protein diet in one period of the menstrual cycle than in another. The failure to induce significant creatinuria by high protein diets is in accord with the findings of M. S. Rose (2) and W. C. Rose (3).

Gamble and Goldschmidt (11) have recently demonstrated that the creatinuria of infants associated with high protein diets derived largely from milk is not due to the protein per se, but probably to the creatine content of the milk ingested. They observed that the quantity

TABLE 3

DATE	SUBJECT S., WEIGHT 56.7 KGM.		
	Creatinine	Creatine as creatinine	Total N
Period I. Low protein diet			
1919	*grams*	*grams*	*grams*
October 24	1.15	0.14	7.55
25	0.97	0.00	5.18
26	1.06	0.00	6.96
27	1.08	0.00	5.45
28	1.02	0.00	6.27
29	1.02	0.00	5.71
30	1.12	0.00	7.09
31			
November 1	1.12	0.00	5.88
2	0.96	0.00	5.43
3	1.02	0.00	5.62
4	1.04	0.00	5.55
5	1.08	0.00	
Period II. High protein diet			
6	1.04	0.00	7.37
7*	1.08	0.00	15.56
8*	0.93	0.00	16.75
9*	1.07	0.00	13.57
10*	1.06	0.00	16.50
11	0.97	0.00	14.26
12	0.99	0.02	20.34
13	0.96	0.06	16.81
14	1.16	0.00	19.60
15	1.04	0.00	14.74
16	0.99	0.00	10.46
17	0.95	0.00	11.20
Period III. Low protein diet			
18	**1.06†**	**0.02**	**12.68**
19	1.05	0.00	10.52
20	**1.15‡**	**0.03**	**8.76**
21	1.17	0.00	8.25

* Menstrual period.

† 1.0 gram creatine per os.

‡ 1.0 gram creatine per os in divided doses.

TABLE 4

DATE	SUBJECT A., WEIGHT 65.0 KGM.		
	Creatinine	Creatine as creatinine	Total N
Period I. Low protein diet			
1919	*grams*	*grams*	*grams*
October 25	1.19	0.0	5.71
26	1.06	0.11	6.99
27	1.24	0.0	6.04
28	1.16	0.0	6.66
29	1.17	0.0	6.15
30	1.19	0.0	6.36
31	0.77†	0.0	
November 1*	1.05†	0.0	
2*	0.91	0.0	4.12
3*	**1.11‡**	**0.05**	**5.73**
4*	0.90	0.0	5.44
Period II. High protein diet			
5	1.12	0.0	4.35
6	1.04	0.0	5.93
7	1.15	0.0	11.08
8	1.07	0.0	10.20
9	1.08	0.0	8.05
Period III. Low protein diet			
10	1.08	0.0	8.62
11	1.13	0.0	9.03
12	1.05	0.0	7.15
13	1.12	0.0	7.50
14	1.17	0.0	7.20
15	1.18	0.0	6.93
16	**1.21§**	**0.31**	**5.64**
Period IV. Milk diet**			
17	1.12	0.10	9.36
18	1.11	0.13	15.38
Period V. Low protein diet			
19	1.02	0.00	9.38
20	1.09	0.00	6.99
21	**1.11††**	**0.16**	**7.26**

* Menstrual period.
† Incomplete collection.
‡ 0.5 gram creatine per os.
§ 2.0 grams creatine and 0.5 gram creatinine per os.
** 3 and 5½ quarts of milk on November 17 and 18, respectively.
†† 1.0 gram creatine per os in 0.5 gram doses.

of whey fed was more directly related to the degree of creatinuria than was the total protein value of the food. We have carried out one study on the effects of diets high in milk on the creatinuria of women. A weighed basal diet low in protein content was ingested during a fore period followed by a period in which casein in the form of "cottage cheese"[1] was added to the basal diet. Protein-free milk was then ingested in amounts equivalent to six quarts of milk, and finally fresh milk and milk powder equivalent to the same quantity of milk (table 6). Although in the high milk period the nitrogen excretion was four

TABLE 5

DATE	SUBJECT R., WEIGHT 51 KGM.		
	Creatinine	Creatine as creatinine	Total N
Period I. High protein diet			
1919	*grams*	*grams*	*grams*
November 7	0.88	0.0	12.21
8	0.91	0.10	13.80
9	0.82	0.07	8.07
10	0.90	0.0	11.52
11*	0.88	0.0	13.28
12*	0.87	0.03	13.86
13*	0.84	0.0	11.36
14*	0.88	0.0	11.32
15	0.92	0.0	9.65
Period II. Milk diet†			
16	0.84‡	0.20	9.30
17	0.89	0.03	12.71
18	0.90	0.0	16.67

* Menstrual period.

† On November 16, 17 and 18, 2, 3 and 5 quarts of milk, respectively.

‡ Diet not strictly meat-free.

times greater than that of the basal diet period, creatine appeared only twice and in small amounts, and disappeared as the high protein diet was continued. Protein-free milk also failed to induce creatinuria. The results are in accord with those of our previous experiments.

Fate of creatine administered per os. The results of enteral administration of creatine to men are somewhat conflicting. According to

[1] The "cottage cheese" was prepared in the laboratory by the precipitation of the casein from fresh skimmed milk by weak acid, and careful washing of the precipitated casein.

TABLE 6

DATE	SUBJECT S., WEIGHT 58.6 KGM.			
	Creatinine	Creatine as creatinine	Total N	Diet
			Period I	
1920	*grams*	*grams*	*grams*	
May 1	1.02	0.0	6.27	Basal
2	1.14	0.0	8.21	"
3*	0.99	0.0	5.97	
4*	0.94	0.0	5.02	
5*	0.95	0.0	5.73	
			Period II	
6*	0.88	0.0	6.85	Basal diet† and casein, 150 grams
7*	0.93	0.0	10.05	Basal diet and casein, 350 grams
8*	1.02	0.0	13.42	Basal diet and casein, 350 grams
9*	0.97	0.0	13.22	Basal diet and casein, 350 grams
10	0.97	0.0	12.72	Basal diet and casein, 350 grams
			Period III	
11	1.06	0.0	11.02	Basal diet and protein-free milk, 150 grams
12	1.14	0.0	8.32	Basal diet and protein-free milk, 180 grams
13	1.10	0.05	8.66	Basal diet and protein-free milk, 180 grams
14	1.09	0.0	8.54	Basal diet and protein-free milk, 180 grams
15	1.02	0.0	7.21	Basal diet and protein-free milk, 180 grams
			Period IV	
16	1.02	0.0	7.41	Basal diet, milk, 1 qt.; and dry milk, 100 grams
17	1.07	0.04	13.55	Basal diet, milk, 1 qt.; and dry milk, 290 grams
18	1.11	0.08	19.18	Basal diet, milk, 3 qt.; and dry milk, 267 grams
19	1.13	0.0	20.70	Basal diet, milk, 3 qt.; and dry milk, 267 grams
20	1.19	0.0	20.40	Basal diet, milk, 3 qt.; and dry milk, 267 grams
21	1.30	0.0	23.68	Basal diet, milk, 4 qt.; and dry milk, 178 grams

* Menstrual period.

† Basal diet. Breakfast: one-half orange, 75 grams bread, 1 cup black coffee. Lunch: 75 grams bread, 2 small slices fat bacon, 100 grams rice, 50 cc. cream. Dinner: 75 grams bread, 150 grams potatoes, 50 grams cheese, 1 egg, 100 grams banana, 1 cup black coffee, 50 grams butter. Total calories approximately 1750. During the periods of casein and of milk, potatoes were omitted from the diet.

Folin (12), in man on a low protein diet creatine in amounts less than 2 to 5 grams is completely retained. In a later publication however Folin and Denis (13) state that "one gram of creatine per day is about the maximum amount which a full grown man can be made to retain, even when kept on a low protein diet and on high protein diets the amounts retained are smaller than on low protein diets." Burns and Orr (14) observed creatinuria after ingestion of only 0.5 gram of creatine. Myers and Fine (15) recovered 16 to 29 per cent of administered creatine when 2 grams or less were fed, while Rose and Dimmitt (16) recovered from 5 to 10 per cent after feeding 1 to 2 grams. We have not been able to find any record of results of creatine administration to women. It is frequently implied (4), (17) that the organism of the female has less power to utilize or convert creatine when ingested than the male, but direct experimental evidence seems to be lacking.

As shown in table 2 with subject S after ingestion of 0.494 gram of creatine during menstruation the excretion of the creatine administered was almost quantitative. An equal amount of creatine fed during the intermenstrual period and in the succeeding menstrual period did not cause creatinuria nor increase the elimination of creatinine. With the same subject (table 3) ingestion of 1 gram of creatine either in one dose or divided into hourly doses of 0.1 gram to facilitate absorption resulted in the elimination of about 3 per cent in the urine. With subject A (table 4) after the administration of creatine in amounts varying from 0.5 to 2.0 grams, creatine was excreted in amounts corresponding to about 10 to 16 per cent of that ingested.

In general the results of these experiments are comparable with the results of similar experiments upon men. With the one exception mentioned above, creatine administered per os to women in amounts up to 2 grams resulted in slight creatinuria only. There seems therefore to be no marked difference in the assimilation and retention of ingested creatine by healthy men and women.

In seeking a possible cause for the excretion of creatine by women, the difference between the results of table 1 and of tables 3 and 4 is suggestive. As previously stated, during the earlier experiments (table 1) both subjects were in poor physical condition, resulting from an unusually hard year of university work. The week of May 10–17 in particular was one of physical and nervous strain, and in both subjects, especially subject A, creatinuria was observed. In contrast with the frequent occurrence of creatine in these experiments, is the infrequent elimination of creatine in the later experiments (tables 2 to 4)

which were carried out during the summer and at the beginning of the college year, when the subjects were in better physical condition. It seems possible also that the creatinuria observed at the beginning of the high protein diet (table 1, subject S, table 6) might have been related to the difficulty of ingestion of such a diet. This is borne out by the gradual disappearance of the creatinuria as the subject became accustomed to the diet. It would be of interest to compare the creatine excretion of healthy women who lead an outdoor life with that of women who work indoors under high nervous tension.

SUMMARY

1. There appears to be no direct relation between the phases of the menstrual cycle and the appearance of creatine in the urine of the adult woman.

2. Protein per se was not the causal factor in the production of creatinuria in normal woman. No evidence was obtained that there was any greater tendency toward the production of creatinuria by high protein diets during the menstrual period than during the intermenstrual period.

3. The results of the administration of creatine per os do not afford any evidence in support of the theory that the power of the adult female organism to destroy or convert ingested creatine is less than that of the male.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

VIII. On the Presence of Vasomotor Fibers in the Vagus Nerve to the Pulmonary Vessels of the Amphibian and the Reptilian Lung[1]

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Numerous attempts have been made in the past to determine whether or not the pulmonary arteries are under vasomotor control. Most of the experimental work has been done on mammals. Because of technical difficulties the results obtained on stimulation of various nerves never gave clear and convincing evidence of the vasomotor control, for the changes in rate of flow through the pulmonary vessels or rise in pressure of the pulmonary artery as a result of stimulation of various nerves could be explained on the rate or strength of heart beat or on changes in the general arterial or venous pressure as a result of the stimulation. So uncertain were most of the results that many of the writers of textbooks do not even treat of the matter as a controversial question but incline to the belief that the pulmonary blood vessels are not under vasomotor control. Others, whom we will mention presently, felt reasonably certain that vasomotor nerves to the pulmonary vessels exist, if it will be conceded that a change in the rate of the blood flow through the isolated and perfused lung as the result of the injection of adrenalin furnishes positive proof that vasomotor nerves supply the pulmonary vessels.

LITERATURE

Our first observations on vasomotor nerves to the pulmonary vessels were made quite accidently during the experimental study of a prob-

[1] A demonstration of the presence of vasomotor nerves in the vagosympathetic to the pulmonary vessels of the frog lung (rana pipiens) was given to the members of the American Physiological Society at the Hull Physiological Laboratory, the University of Chicago, December 29, 1920.

lem of an entirely different nature. Since the results were so clear-cut and decisive we concluded that we could present data on pulmonary vasomotor nerves which were, because of the fortunate experimental conditions employed by us, quite striking and original. After we had made an extended search of the literature it became evident that many of the previous investigators and writers on this subject failed to present all of the evidence to their readers. Certainly, some of the most convincing experimental work on the subject had been overlooked by some and discredited by others, without going to the trouble of repeating it. We therefore felt that a brief chronological survey of the previous work was desirable before the presentation of our own results.

It seems that even before Claude Bernard and Brown-Séquard had demonstrated by their classical experiments the presence of vasoconstrictor nerves in the cervical sympathetic (1851) Moritz Schiff (22) noted, in 1847, a congestion of the lungs following double vagotomy which was quite different from the congestion and consolidation of the lungs of pneumonic animals. On the basis of our present knowledge his observations might be interpreted as a congestion due to the release of the pulmonary vessels from the tonic influence of vasoconstrictor nerves carried by the vagus. At any rate, the observation is interesting since it was made on the very vessels which even after the discovery of vasomotor nerves have been supposed, on the basis of experimental studies, to possess few if any vasomotor nerves of physiological consequence. In two long papers (22), (23) Schiff, on the basis of numerous and ingenious experiments coupled with accurate observations, ably defended his position against Traube in maintaining that double vagotomy effects a hyperemia of the lung which is quite different from the aspiration pneumonia which may secondarily be engrafted on the hyperemic condition, if operative procedures are not instituted to prevent it. Traube, among others, never recognized the "neuroparalytic congestion" of the pulmonary blood vessels described by Schiff following double vagotomy. On the discovery of vasomotor nerves some three years later Schiff interpreted his "neuroparalytic congestion" of the lungs in terms of a dilatation of the pulmonary vessels because of section of tonic vasoconstrictor nerves contained in the vagus of the pulmonary vessels. Forty-four years after the appearance of his first paper (24) (1894) on neuroparalytic pulmonary congestion he devised experiments to demonstrate by direct stimulation vasoconstrictor control of the pulmonary vessels through the vagus nerve. His failure to satisfactorily prove their existence in the mammal can probably be explained by a

misguiding procedure in the preparation of his animals which will be discussed more fully presently.[2]

No further work on vasomotor nerves to the pulmonary vessels is reported until Brown-Séquard (4) reopened the investigation in 1871–1873. As a result of his work physiologists focussed their attention on the thoracic sympathetic nerves as the vasomotor nerves to the pulmonary vessels; for Brown-Séquard failed to obtain any vasomotor effects on stimulation of the vagus nerve after atropinization of the animal. In 1874, Genzemer (29) confirmed the findings of Schiff and extended the work chiefly in these two directions: *a*, he maintained that the congestion of the lungs after double vagotomy appears within several hours after the operation; and *b*, stated that on exposing the parietal pleura by removal of the intercostal muscles it was possible to see with great plainness a marked reddening of the lung immediately following section of both vagi nerves (rabbits). The author concludes that tonic vasoconstrictor nerves for the pulmonary nerves are present in the vagi and that an aspiration pneumonia is a condition which often supervenes as a *secondary* phenomenon following double vagotomy. A few years later Lichtheim (18) likewise eliminated the vagus nerve as a possible vasomotor nerve to the pulmonary vessels when he failed to observe any change in the pressure of the pulmonary artery following stimulation in animals *atropinized* in order to eliminate the usual cardio-inhibitory effects incident to such stimulation. Since he obtained an increased pressure in the pulmonary artery following direct stimulation of medulla oblongata, faradization of the cervical cord, asphyxia and strychnine he concluded that the vasomotor nerves run down the cord and reach the lungs by way of the thoracic sympathetic chain rather than by way of the vago-sympathetic, a conclusion, as we have seen, previously arrived at by Brown-Séquard. In support of this position François-Franck (10) showed conclusively that stimulation of the thoracic sympathetic nerves to the lungs is followed by a *rise* in the pressure of the pulmonary artery synchronous with a *fall* in the pressure in the left auricle. He likewise concluded that the vagus contained no vasomotor fibers for the pulmonary vessels; for stimulation of the vagus nerves in animals *atropinized* to eliminate the disturbing cardiac effects had not the slightest effect on the pressure in the pulmonary artery or left auricle.

[2] Schiff finally came to the conclusion, in 1894, that the vagus carried vasoconstrictor fibers to the bronchial arteries and that the branches of the pulmonary artery received vasoconstrictor branches from the thoracic sympathetic nerves.

No one remembered or considered seriously the neuroparalytic congestion or hyperemia of the lung following double vagotomy (chiefly in rabbits) when Brown-Séquard and those who followed him demonstrated that stimulation of the vagus in animals prepared by injection of atropine to eliminate cardio-inhibitory effects was without the slightest effect on the pulmonary vessels. Nor did anyone take particular note of or seriously consider (except Schiff in 1894) two short articles by Couvreur (8), (9) which appeared in 1889. In these articles Couvreur stated that if the peripheral vagus trunk of a frog is stimulated after sectioning the cardiac fibers, one can under the microscope see a complete cessation of the blood flow through the lung.[3] He attributed these effects to the sympathetic fibers contained in the peripheral vagus trunk on the basis of the previous work of Brown-Séquard and François-Franck on the atropinized mammal; and hazarded the guess that the hemorrhages into the lung following double vagotomy might be explained on the basis of vasoconstrictor nerves carried in the vagus of some mammals. He had seen such effects one-half hour after double vagotomy in guinea pigs. It seems that he was not familiar with the similar views of Schiff outlined above and published 32 years previously.

Last year Ozorio de Almeida (30) reported a fatal congestion and edema of the lungs of double vagotomized guinea pigs following the stimulation of the peripheral ends of the vagus nerves. Double vagotomy alone without stimulation of the nerves did not effect an edema.

In the year 1890 Arthaud and Butte (1) stated that the vasoconstrictor fibers in the trunk of the vagosympathetic nerves of the frog described by Couvreur were in tonic activity; for these two investigators found that section of the vago-sympathetic nerve on one side led to a marked vasodilatation of the vessels of the corresponding lung.

Among others, Cavazzani (7) was not impressed by the statements of Couvreur and Arthaud and Butte; for the following year he refused to accept the assertion or the experimental evidence on the basis of the work reported by the physiologists mentioned above, that even light excitation of the vagus in frogs gave constriction sufficient to completely arrest the circulation through the lung. Cavazzani (1891) was apparently the first who investigated the vasomotor innervation of the

[3] When making the original observations on the frog and the turtle we were not acquainted with the work of Couvreur, Arthaud and Butte, and Krogh. As a matter of fact all of our experimental work was completed before we instituted a search of the literature which is presented in this section.

lungs by the perfusion method. Perfusing the lungs under constant pressure and then stimulating various nerves, he found that the sympathetics contained dilator and that the vagus contained constrictor nerves to the pulmonary vessels. Stimulation of the vago-sympathetic in the dog was usually followed by dilatation, however. Two years later Henriques (15) reported finding a rise in the pressure of the pulmonary artery accompanied by a fall in the pressure of the left auricle following stimulation of the vagus of a curarized cat. Since similar observations in dogs and rabbits effected a drop in arterial pressure of the pulmonary artery with rise in the left intra-auricular pressure with no change in the rate of the heart he concluded that the vagus contains chiefly dilator fibers for the pulmonary vessels of the dog and rabbit and constrictor fibers to the pulmonary vessels of the cat. This conclusion for the cat was drawn from a single experiment and the author himself questions its validity. Parenthetically, we might call the reader's attention to the fact that Henriques did not atropinize his animals prior to vagus stimulation. His work was reviewed the following year by Bradford and Dean (2) who measured simultaneously the pressure in the pulmonary and carotid arteries in morphinized, curarized, and doubly vagotomized dogs under chloroform anesthesia whilst stimulating various anterior roots of the dorsal cord within the spinal column. They obtained evidence of the presence of vasomotor (specifically vasoconstrictor fibers) for the pulmonary vessels in the 3rd, 4th and 5th dorsal nerves. They also attempted to elicit reflex constriction of the pulmonary vessels by stimulation of the intercostal, sciatic, etc., nerves. They concluded that the vasomotor system of the pulmonary vessels is but poorly developed. A search for the presence of vasomotor fibers in the vagus was unsuccessful. Following the lead set by previous investigators these authors also injected *atropine* to eliminate the cardiac effects of the vagus on the heart. No mention is made of the work of Couvreur; but the work of Henriques is mentioned as a unique example of the only author who ascribed vasomotor action to the vagus for the pulmonary vessels. During the following two years, François-Franck (11), (12), (13), (14) extended and strengthened the position held by him since the appearance of his first work in 1881. He measured simultaneously the pressure in a branch of pulmonary artery, the aorta, the left auricle and took plethysmographic records of the lung while stimulating the thoracic sympathetic trunk at various points or first thoracic nerve. He confirmed the work of Bradford and Dean in every particular. Among other things he showed that vasoconstriction

of the lung following stimulation of the sympathetic nerves was accompanied by an increase in the lung volume of the affected side together with a decrease in the left intra-auricular pressure. The increase in volume of the lungs under these conditions he attributed to the increased efficiency of the ventricular systole. By his method he demonstrated reflex vasoconstriction of the pulmonary vessels resulting from stimulation of the central ends of the crural, sciatic, and central abdominal vagus (from the latter after atropinization to prevent reflex cardiac inhibition). In fact, every afferent nerve (cutaneous or visceral) which he tried gave him the phenomenon. He considered the vasoconstriction in the pulmonary vessels as possible mechanism useful under certain conditions in preventing the arterial pressure from becoming too high by limiting the amount of blood going to the left ventricle. The matter received no further attention aside from a review of the literature on the pulmonary circulation by Tigerstedt in 1903 (27). In this review the work of Couvreur is not mentioned in the text though the bibliography includes a reference to it. Considering that an account of Couvreur's work appeared in his monograph on the physiology of the circulation published 10 years earlier (26), it would appear that Tigerstedt was not impressed with its accuracy or importance.

From this time on investigations have been restricted chiefly—almost exclusively so—to perfusion experiments of the isolated lung with a drug whose action is generally considered to be on vasomotor nerve endings, namely, adrenalin. Brodie and Dixon (3) perfused the isolated lungs of cats with cats' own blood, for they found that when they diluted the blood with Ringer's solution edema of the lungs soon supervened. In half of their experiments the lung was inflated rhythmically. The lung was perfused through the pulmonary artery. Another cannula tied to the left auricle collected the blood which issued from the perfused lung, the other lung having been tied off. As a control on the viability of the preparation under the conditions of experimentation the same methods were employed on other organs whose vessels possess vasomotor nerves easily demonstrable under better physiological conditions. These investigators failed to obtain evidence of any vasomotor control over the pulmonary vessels on stimulation of the spinal cord, thoracic sympathetic chain, loop of Vieussens, inferior cervical ganglion, vagus nerve, or the base of lung itself although under identical conditions stimulation of nerves to the hind legs or intestine gave marked evidence of vasoconstriction of the vessels of these organs. Even adrenalin was without effect in the dosage used, whereas the same dosage

produced the anticipated results in the vessels of the limbs and intestines. Pilocarpine and muscarine, which are considered universally as stimulants to nerve terminals, caused contraction of the systemic vessels but relaxation of the pulmonary vessels. They concluded, therefore, that the pulmonary vessels are devoid of any significant number of vasomotor fibers. The same year Plumier (20) confirmed some of the findings of François-Franck and furthermore stated the vasoconstrictor action following stimulation of the loop of Vieussens is bilateral; for he described a rise in the pressure of the *right* pulmonary artery with a drop in the left intra-auricular pressure on stimulation of the left sympathetic nerve (dogs). In a second paper Plumier (21) described experiments of the same nature and performed under conditions similar to those of Brodie and Dixon. In his experience stimulation of the annulus of Vieussens always caused vasoconstriction of the pulmonary vessels as did injections of adrenalin. Plumier emphasized the importance of using fresh defibrinated blood without diluting it with Ringer's solution. He furthermore suggested that the solutions of adrenalin used by Brodie and Dixon (1:20,000 in cc. doses) were too weak. Plumier used from 0.2 to 0.5 mgm. adrenalin as a dose. This dose caused enormous vasoconstriction preceded by a vasodilatation. He does not call attention to this primary dilatation. Plumier concluded that the vasomotor nerve endings in the lung were less sensitive to adrenalin than those of the leg for he could never completely stop the flow through the lungs by its use as he could by perfusion of the drug through the vessels of the leg. Some years later, Langendorff (17) showed that adrenalin caused constriction in the isolated branches of the pulmonary artery of the sheep, pig, calf and cat. This constriction was more powerful (if the tracing submitted is representative of all his experiments) than the constriction obtained on direct stimulation of the arterial wall itself with a tetanizing current.

Krogh in 1907 (16) made the following observations on the vasomotor control in the lungs of the turtle. The blood was rendered non-coagulable by hirudin and a Y-cannula placed in the pulmonary vein on one side, so that the return of the pulmonary vein blood could be temporarily prevented and the blood collected in a receptable for measurement of the blood-flow rate per unit of time. Records were also taken of the general arterial pressure. With this method of investigation Krogh found *1*, that the rate of the blood flow through the lung varied independently of the variation in the general arterial pressure; *2*, that on section of the vago-sympathetic nerve in the neck there was a great in-

crease in the rate of the blood flow through the lung of the same side, and subsequent variations in the blood flow through this denervated lung ran parallel with the changes in the general arterial pressure; *3*, that on section of the vago-sympathetic nerve on one side the rate of the blood flow through the lung of the opposite side was reduced.

Krogh concluded that the vago-sympathetic nerves in the turtle contain vasomotor nerves to the lung blood vessels, and that these (the vasoconstrictors) are in tonic activity.

The only objection that may be raised against Krogh's observations and conclusion is that the state of contraction of the lungs was not controlled.

In 1909 Wiggers (28) published experiments which essentially confirm the earlier work of Plumier.

As a general recapitulation we might state that before the discovery of vasomotor nerve fibers of any kind, Schiff (1847) suggested on the basis of experimental results that the vagi nerves carried fibers to the blood vessels of the lungs and that division of these fibers effected a neuroparalytic hyperemia of the lungs. Subsequent attempts to detect vasomotor fibers for the pulmonary blood vessels in the vagi of mammals failed except in two instances: Henriques (1893) on the basis of but few experiments definitely decided on their presence as did Schiff (1894). Henriques did not use atropine to eliminate the inevitable confusing cardiac inhibition which would itself markedly diminish or totally stop the flow of blood through the pulmonary vessels; Schiff used it to some extent but warns investigators not to use the drug in large quantities on the basis of his experience with it. All other investigators before Henriques and since then who failed to detect any vasomotor fibers in the vagus for the pulmonary vessels have without exception used this drug. (Brown-Séquard, 1871; Lichtheim, 1876; François-Franck, 1880; Bradford and Dean, 1894.) On the basis of such decisive negative evidence, Couvreur's (1889) description of vasomotor fibers for the pulmonary vessels in the vagus of the frog (really vasosympathetic) was most generally overlooked by some investigators, openly discredited by one investigator (Cavazzani, 1891), and slowly relegated to oblivion by another (Tigerstedt, 1903). That these vasoconstrictor nerves in the vagus were in constant tonic activity as stated by Arthaud and Butte (1890), received even less consideration by subsequent writers and investigators. Accordingly, later physiologists disregarded the vagus and looked for vasomotor fibers in the sympathetic nerves to the lung and found them (Bradford and Dean, 1894;

François-Franck 1891 and 1895; Plumier, 1904). Brodie and Dixon alone denied their presence (1904). Bradford and Dean (1894) and François-Franck (1896) even described experiments in which reflex pulmonary vasoconstriction was effected by the stimulation of various afferent nerves (visceral and cutaneous). Krogh (1907 and 1910) presented undubitable evidence that the vago-sympathetic nerve in the turtle contains tonic vasoconstrictor action over the pulmonary vessels. No cognizance of this work appears to have been given by any writer except Stewart (25). With the discovery of the specific hemodynamic properties and mode of action of adrenalin, the problem was again investigated by perfusing the surviving mammalian lung with blood or a blood-Ringer mixture and noting the effect of adrenalin on the rate of flow through the lung. The results, on the whole, were positive (Plumier, 1904; Wiggers, 1909) in that such perfusions reduced the rate of flow through the lung.

But this method does not decide whether the vagus or the sympathetics carry the vasomotor fibers. The assumption that the drug, adrenalin, stimulates only the myoneural junctions of the sympathetics, is in the case of our experiments, quite unwarranted; for if we can show that the vagus carries vasoconstrictor nerves to the pulmonary vessels of the frog and turtle and that these are *paralyzed by atropine*, it might be possible to explain the constrictor action of adrenalin on the blood vessels of the frog's and turtle's lung by its stimulating action on the myoneural junctions of the vagus. At any rate, from our experience with atropine on the frog and turtle it would seem highly probable that previous investigators have failed to detect the vagus vasomotor fibers for the pulmonary vessels in the mammals by paralyzing the nerve with this drug before stimulating, except Cavazzani (1891) who, using the perfusion method, described vasoconstrictors in the vagus of rabbits but dilators in the vago-sympathetics of dogs.

METHODS

Experiments on frogs. In all cases the animals were decerebrated. No anesthetics were employed. Moderate doses of curare were used in some preparations. In other cases the animals were immobilized by section of the spinal cord below the medulla and pithing it posteriorly. The animals were fixed dorsal side down, and the lungs exposed by a ventral median incision extending from the pubis to the angle of the jaw. In nearly every case the lung under observation was provided

with a cannula in the tip for recording the intrapulmonic pressure and where the experiments involved section of the vago-sympathetic nerve, the glottis was closed by forceps prior to this section, as described in our first paper on lung automatism and lung reflexes in the amphibia (5).

1. Changes in the tonus of the pulmonary blood vessels on section of the cervical sympathetic and the vagus nerves. With the glottis closed and the lungs moderately distended by inflation with air, the cervical sympathetic nerve was isolated and sectioned between the brachial plexus and the root of the vagus, the vago-sympathetic nerve was sectioned in the neck, and the changes in caliber of the pulmonary arteries produced by these sections determined by direct inspection of the lung. The section of these nerves may induce change in the caliber of the pulmonary arteries indirectly through changes in the heart rate and general arterial pressure. These factors would operate equally on the intact and the denervated lung. Hence, in this series of experiments, the blood vessels of the intact lung served as a control of the cardiac and arterial blood pressure factors.

2. Changes in the tonus of the pulmonary arteries on stimulation of the peripheral end of the cervical sympathetic and the vagus nerves. The changes in the caliber of the pulmonary vessels were determined by direct inspection. The cardiac and the arterial blood pressure factors were controlled in the following ways: *a*, tying of the heart at the sinus or excision of the heart; *b*, paralysis of the cardiac vagus with moderate doses of nicotine or curare, and recording the arterial pressure from a branch of the left aorta, in case the right lung was used for observation, or vice versa. The factor of variations in the lung tonus was controlled by moderate doses of nicotine (intravenously) or dilute solutions of atropine applied to the surface of the main pulmonary arteries. It has been shown by us previously that nicotine reverses the effects of vagus stimulation on the lung motor tissues. Hence by the use of this drug it is possible to observe the influence of the vagus stimulation on the pulmonary arteries parallel with either contraction or dilatation of the lung musculature. In the frog the main branches of the pulmonary artery run on the surface of the lung. Dilute solutions of atropine applied directly to the pulmonary artery paralyze the vagus vasomotor fibers before the lung motor fibers, so that the purely mechanical effects of lung contractions on the caliber of the pulmonary arteries may be studied.

3. Perfusion of the lung. This method was used for the study of the action of epinephrin and other drugs on the pulmonary blood vessels as

well as the influence of the stimulation of the vago-sympathetic nerve on these vessels. In these experiments the lung was left in situ, the glottis being closed and a cannula being fixed in the tip of the lung for recording the intrapulmonic pressure. The right lung was used. The arterial cannula was tied in the right aorta and all branches of this aorta ligated, save the right pulmonary artery. The main pulmonary vein, even in the common frog, is large enough for insertion of a cannula, but anatomical relations and mechanical conditions render this method impossible. For that reason we inserted the cannula for the venous outflow through the auricle into the sinus venosus. The ventricle being excised and the left aorta ligated, the only fluid entering the sinus is that from a pulmonary vein from the right side. This method of perfusion of the frog's lung works very satisfactorily, and when proper mechanical adjustments are made the drop method of venous outflow becomes a very delicate indicator of the tonus of the pulmonary vessels.

The chief difficulty lies in securing a physiological substitute for the frog's blood. Pure Ringer solution will not do. When pure Ringer's solution is perfused through the frog's lung, even under very moderate pressure, the lung passes into extreme tetanus or hypertonus within a few seconds, and in a few minutes the lung becomes edematous (as noted by previous investigators) and the lung cavity fills up with Ringer's solution and mucus. The nerves both to the lung motor tissues and to the blood vessels are quickly paralyzed. We found it necessary to add $\frac{1}{4}$ to $\frac{1}{2}$ defibrinated frog's blood to the Ringer solution in order to keep the lung tissues approximately normal for 20 to 30 minutes of perfusion.

The drugs were added to the perfusion fluid close to the inflow cannula in the aorta, and without change in the perfusion pressure. The mechanical influence from the lung contractions or relaxation following the vagus stimulation were again controlled by appropriate doses of nicotine.

4. The influence of the body temperature of the frog. The work on the frog was begun in the summer of 1920 and resumed in November and December of the same year. Frogs as secured from the dealers in this commodity vary greatly in vigor, depending probably on the length of time in captivity and the care in handling. We noted that the frogs kept in running water (8°–10°C.) during the month of December exhibited as a whole much feebler vasomotor responses in the lungs than did the frogs worked on during the summer. We therefore resorted to the expedient of slowly warming the frogs to approximately room tem-

perature (20°–25°C.) before the experiments. Raising the body temperature in this manner appeared to increase the intensity and rapidity of pulmonary vasomotor action.

5. Mechanical spread of excitation current from the vagus to the pulmonary blood vessels. We noted early in the work that the weak tetanizing current applied to pulmonary artery at the base of the lung causes extreme contraction of the artery throughout its whole length. Unless special care is taken on tetanization of the peripheral vagus there may occur an escape of the current to the carotid and by muscular conduction down the pulmonary artery. This source of error was checked up by tetanization of the carotid isolated from the vagus. The stimulation of the carotid does not change the caliber of the pulmonary arteries.

Experiments on turtles. All animals were decerebrated. No anesthetics were used, but in some cases the preparations were partially or completely curarized.

1. Changes in tonus of the pulmonary blood vessels on section of the vagi nerves in the neck. These were investigated in two ways. The lungs were isolated from the dorsal side, and the bronchi or the trachea ligated after moderate inflation of the lungs with air. In such preparations the change in caliber of the pulmonary vessels in each lung following section of the respective vagus nerve was determined by direct inspection of the lung. In another series we perfused one lung in situ, with Ringer plus defibrinated blood, care being taken in fixing the cannulae in the pulmonary artery and vein to leave the pulmonary branches of the vagus intact. The rate of blood flow through this lung was then recorded by the drop method, parallel with the record of the intrapulmonic pressure so as to control the mechanical factor involved in lung contractions and relaxations. Section of the vagus in such preparations yield objective evidence of change in the caliber of the lung blood vessels.

2. Changes in the caliber of the lung arteries on stimulation of the peripheral end of the vagus nerve. This was investigated in the following way: *a*, The respective lung was isolated from the dorsal side except for the pulmonary nerves and blood vessels, the bronchus ligated; the vagi branches to the heart sectioned or paralyzed by intravenous injection of nicotine; arterial blood pressure recorded from the central end of the pulmonary artery of the opposite side. In such preparations the effects on the pulmonary blood vessels produced by tetanization of the peripheral vagus were determined by direct inspection of the lung. In most of the preparations in this series the animals were fixed ventral side down, the plastron left intact and the lungs isolated from the dor-

sal side. In a few preparations the plastron was removed and the lungs isolated from the ventral side, in order to be sure that the possible pulmonary branches of the cervical sympathetic nerve would be left intact. These preparations were used particularly to determine the influence of the sympathetic nerves on the pulmonary arteries.

b. One lung was isolated and perfused with a Ringer-turtle blood mixture in situ, and the effects of vagi stimulation on the caliber of the pulmonary vessels determined by the change in rate of the venous flow. No attention was paid to the cardiac action of the vagus stimulation, as this could not influence the perfused lung.

c. The lung was isolated, excised, and perfused with the Ringer-blood mixture under constant pressure. The bronchus was ligated and a cannula fixed in the tip of the lung for recording the intrapulmonic pressure. During the perfusion the preparation was kept moist with Ringer solution. The drop method of venous flow recorded the influence of vagus stimulation on the pulmonary vessels, and the mechanical factor of the lung contraction controlled by inflation of the lung to a pressure equal to or exceeding that induced by tetanization of the vagus.

3. The action of epinephrin and other drugs on the pulmonary vessels. Most of the experiments in this series were made by perfusing the isolated lung (excised or in situ) with a Ringer blood mixture under constant pressure, while taking parallel records of the intrapulmonic pressure (lung tonus or contractions). In a few cases the drugs were injected intravenously and the action on the pulmonary vessels determined by direct inspection of the exposed lung, parallel records being taken of the intrapulmonic pressure and the general arterial pressure.

The drugs were introduced into the perfusion fluid near the cannula fixed in the pulmonary artery.

4. The reflex control of the pulmonary vasomotor mechanism. Only a few experiments were made on this phase of the problem. In all cases the lung under observation was isolated from the dorsal side and prepared for recording of the intrapulmonic pressure and the general arterial pressure, care being taken not to injure the pulmonary branches of the vagus nerve. Various afferent nerves were then stimulated and the effects on the lung blood vessels noted by direct inspection. In other preparations the lung, prepared as above, was perfused with the Ringer-blood mixture, the reflex effects from sensory stimulation on the lung arteries recorded by the rate of the venous outflow.

For a more detailed account of our method of preparing the turtle for observing and recording the lung rhythms, the reader is referred to an earlier paper in this series (6).

RESULTS ON THE FROG

1. The effects on the tonus of the pulmonary arteries of ligation of the vagus and the cervical sympathetic nerve. When the frog is prepared and the lungs exposed for the study of the lung automatism and the lung reflexes, as described by us in an earlier report, the pulmonary arteries on the two lungs show usually an equal degree of dilatation, as nearly as this can be determined by direct inspection. Occasionally the arteries of one lung are more constricted than on the opposite lung, and in some preparations there is periodic variation in the size of the lung arteries, like Traube-Hering waves of blood pressures. These periodic variations in the caliber of the lung arteries may or may not be synchronous in the two lungs. Blood pressure records were not taken in these animals. Under these conditions the periodic variations in the size of the pulmonary arteries that were synchronous in the two lungs could have been due to changes in the strength of the heart beat and consequent changes in the arterial pressure, that could not be detected by the method of direct inspection. It was not due to changes in the rate of the heart. But this explanation fails to account for the tonus variations in the arterial vessels that were not synchronous in the two lungs. In many of the preparations the glottis was closed and graphic records taken of the lung tonus and the lung contractions. In this way we determined that the periodic changes in the caliber of the pulmonary arteries were independent of the tonus changes in the lung musculature.

In these preparations ligation of the cervical sympathetic nerve had in no instance any influence on the caliber of the pulmonary arteries either on the same or on the opposite side. But *ligation or section of the vagus nerve usually caused, on the same side, a temporary constriction of the lung arteries followed by a permanent dilatation.* The temporary vasoconstriction was noted less frequently than the permanent dilatation. It is obvious that both the temporary constriction and the permanent dilatation of the arteries had to be marked in order to be made out with a certainty by direct inspection. In some of our preparations the permanent dilatation of the lung arteries following the ligation of the vagus nerve on the same side was as marked as the hyperemia of the ear after section of the sympathetic nerve in the rabbit.

It should be noted that ligation of the vagus produces the above effects on the pulmonary vessels of the same side only. The fact that the caliber of the arteries of the intact lung is not influenced by section of the vagus on the opposite side eliminates the heart and the blood

pressure as factors in the vasodilatation of the denervated lung. But ligation of the vagus induces a permanent hypertonus of the lung on the same side. Before we may conclude on the basis of these experiments that the vagus contains vasoconstrictor fibers to the lung and that this vasoconstrictor mechanism is in tonic activity, it is necessary to show that the vasodilatation is not an effect of the lung hypertonus. It is conceivable that the lung contraction may induce dilatation of the lung arteries as a mechanical effect of capillary compression and hence increased resistance. Or, the vasodilatation may be a type of peripheral physiological coördination, the greater muscular work in the lung effecting local vasodilatation by diffusion of tissue metabolites. The complete answer to these possible explanations is secured by another line of experiments. But the following facts may be stated here. In some preparations the ligation of the vagus caused hypertonicity of the lung but no vasodilatation that could be detected by direct inspection. This shows that the pulmonary vasodilatation is probably not due to lung hypertonus, as the latter may be induced without the former.

The obvious conclusions from these experiments seem to be that the vagus (but not the sympathetic) contains pulmonary vasoconstrictor fibers, and that this mechanism is in tonic activity. The degree of this tonic activity will naturally vary with the condition of the frog, the trauma of the operation, degree of asphyxia, etc.

2. The influence on the pulmonary arteries of stimulation of the peripheral end of the vagus and the sympathetic nerves. This was determined by direct inspection, and by the rate of venous flow in the perfused lung. In the case of the first method the cardiac factor was controlled by curare or nicotine.

Typical tracings illustrating the results obtained by the first method are reproduced in figures 1 and 2. It will be noted that tetanization of the peripheral vagus (after nicotinization) induces complete constriction of the lung arteries, with no change in pulse and arterial pressure or with a synchronous acceleration of the heart and rise in blood pressure (stimulation of the accelerator nerves), and independent of the lung contraction and lung inhibition. These experiments eliminate the heart, the blood pressure and the lung tonus as factors in pulmonary vasoconstriction. We are obviously dealing with a true vasomotor nerve mechanism. In good preparations the obliteration of the pulmonary arteries on tetanization of the peripheral vagus is as complete as the blanching of the ear on stimulation of the cervical sympathetic

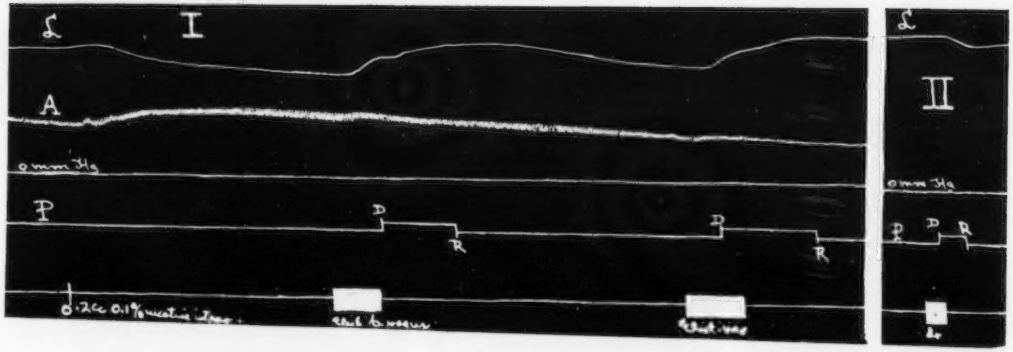

Fig. 1.[4] Record of the intrapulmonic pressure of the right lung, *L*, left aortic blood pressure, *A*, constriction of the pulmonary vessels, *P*, where at *D* the pulmonary vessels disappeared completely to return with maximum pulsation at *R*, as a result of stimulation of the peripheral end of the vagus after intravenous injection of 2 mgm. nicotine as indicated by the signal magnet tracing of the lowermost line. Frog decerebrated, spinal cord transected below the medulla and pithed. Intrapulmonic pressure from tip of right lung; glottis closed with hemostat.

Showing in I a complete *disappearance* of the pulmonary vessels as a result of stimulation of the peripheral end of the vagus and the subsequent reappearance of the pulmonary vessels at a time when the aortic pressure and rate of heart beat were quite steady and when the intrapulmonic pressure was considerably higher than at any period prior to the vagus stimulation.

Showing in II complete blanching of the blood vessels of the lung of the same frog on vagus stimulation with arterial pressure at zero because of excision of the heart at a time when the lung showed inhibition. These tracings are offered to illustrate the complete independence of the cardiac, pulmonary and vasomotor effects on stimulation of the peripheral end of the vagus.

[4] The figures in this article have been reduced from one-half to one-fourth their original size.

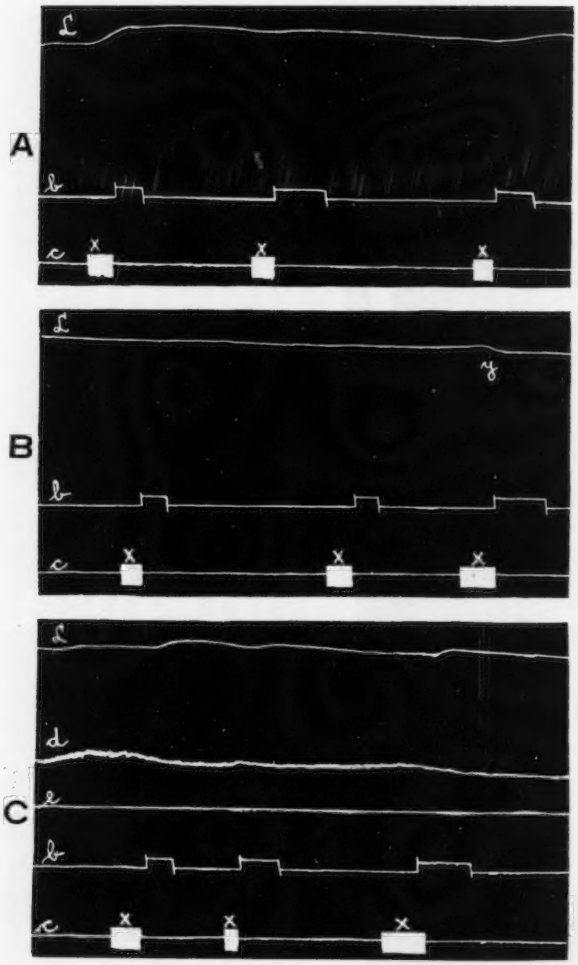

Fig. 2. Simultaneous records of the intrapulmonic pressure, constriction of the pulmonary arteries and general arterial blood pressure of a frog on tetanization of the peripheral end of the vago sympathetic after the intravenous injection of 2 mgm. nicotine. Frog decerebrated, spinal cord transected below the medulla and pithed. Cannula in tip of right lung, glottis closed with mosquito forceps. Intravenous injection through the abdominal vein. Blood pressure from left

nerve, in the mammal (rabbit, cat). By our method the vagus-lung vasomotor mechanism is as readily demonstrated as the vasomotor nerves to the ear.

All of our experiments attempting to influence the tonus of the pulmonary blood vessels by stimulation of the peripheral end of the cervical sympathetic nerve yielded negative results. Since stimulation of the cervical sympathetic resulted in cardiac acceleration without a vasoconstriction of the pulmonary vessels, it is reasonable to assume that the vasomotor nerves were not injured in the isolation of the sympathetic. The pulmonary vasomotor fibers in the vago-sympathetic nerve belong therefore essentially to the vagus system. If the cervical sympathetic nerves contain any vasomotor fibers to the pulmonary vessels our method of direct inspection fails to reveal them.

Tracings showing the effect of vagus stimulation on the flow of blood through the perfused lung are reproduced in figures 3 to 5. It will be noted that tetanization of the peripheral vagus retards or stops the blood flow through the lung, irrespective of whether the stimulation induces an increase or a decrease in the lung tonus. The pulmonary vasomotor mechanism thus works independently of the lung motor mechanism. In fresh preparations tetanization of the vagus causes sufficient vasoconstriction to stop completely the blood flow through the lung. But this vasomotor mechanism fails quickly in the perfused preparations.

aortic arch. In all tracings L = record of intrapulmonic pressure; b = signal magnet tracing in which the upstroke indicates the *complete* constriction of the pulmonary arteries as determined by direct observation and the downstroke their complete reappearance; c = signal magnet tracing indicating at x stimulation of the peripheral end of the vagus; d of tracing C is a record of the aortic pressure, e being the 0 mm. Hg. pressure.

In A the constriction of the pulmonary vessels on stimulation of the vagus at x occurs during or is associated with a contraction of the pulmonary musculature.

In B the complete vasoconstriction on vagus stimulation occurs without any significant change in the intrapulmonic pressure or even with a slight inhibition of the lung, y, together with a cardiac acceleration.

In C stimulation of the peripheral vagus at x caused a complete constriction of the pulmonary vessels accompanied in several instances by slight rise in the aortic pressure (cardiac acceleration) and a slight rise in the intrapulmonic pressure, which in every instance comes on after the vasoconstriction is complete and outlasts the vasoconstriction.

Showing the independence of the cardiac, the vasoconstrictor and lung motor action of the vagus.

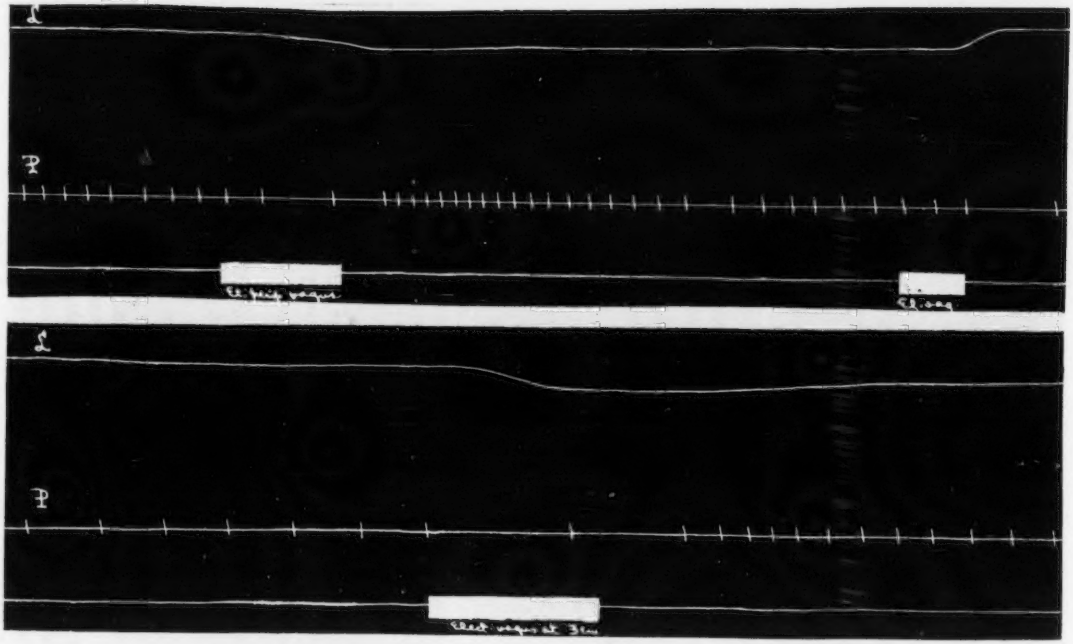

Fig. 3. Record of the intrapulmonic pressure of the right lung of frog, *L*, with signal record recording drops of blood-Ringer mixture issuing from the same lung, *P*, with lowermost signal magnet tracing indicating electrical stimulation of the peripheral end of the right vagus. Frog decerebrated, spinal cord transected below the medulla and pithed. Perfusion of the right lung through left aortic arch, all vessels other than right pulmonary artery (including the bulbus aortae) having been tied off. Outflow from the lung measured in drops of fluid issuing from a cannula tied in the auricle.

Showing a diminution in the outflow from the perfused lung on stimulation of the vagus followed by a temporary acceleration associated either with a contraction or inhibition of the lung.

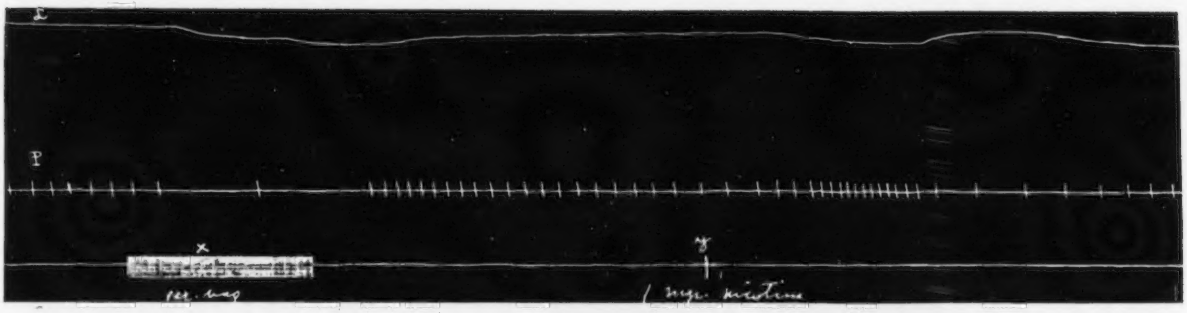

Fig. 4. Record of the intrapulmonic pressure, L, of the right lung of frog with record by means of signal magnet, P, of drops of blood-Ringer mixture issuing from the same lung with lowermost line indicating electrical stimulation of peripheral end of the right vagus at x and the perfusion at y of the same lung with 1 mgm. of nicotine.

Animal prepared as described in the text and in legend of figure 3.

Showing extreme diminution in rate of outflow from the lung on electrical stimulation of the vagus associated with an inhibition of the lung; also primary acceleration and, secondarily, a marked diminution in the rate of outflow as the result of the perfusion of the lung with nicotine.

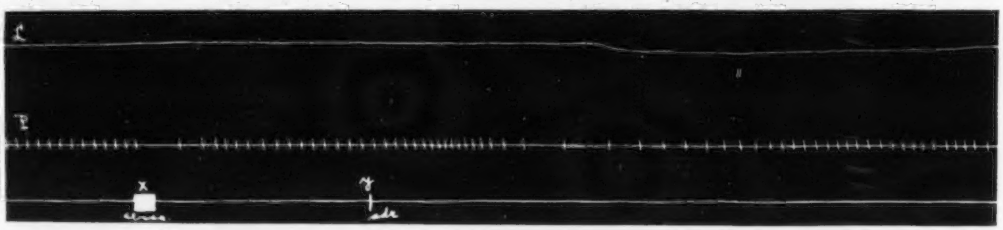

Fig. 5. Record of the intrapulmonic pressure of the right lung of the frog, L, with signal magnet record indicating drops of blood-Ringer mixture issuing from the same lung, P, and lowermost line indicating stimulations of the peripheral end of the vagus at x and the perfusion of the lung at y with $\frac{1}{10}$ cc. 1:1000 adrenalin chloride solution. Animal prepared as described in the text and in the legend of figure 3.

The record shows temporary diminution in rate of flow from the lung on stimulation of the vagus as well as primary increase, and then marked diminution in the rate of outflow as the result of the perfusion of the lung with adrenalin chloride in the absence of any significant changes in the intrapulmonic pressure.

In none of our preparations and with no strength or type of stimulation of the peripheral vagus did we obtain primary dilatation of the pulmonary blood vessels. This does not necessarily mean that vasodilator nerves are absent. It does mean, at least, that the constrictor mechanism is preponderant.

3. The effect of epinephrin and histamine on the pulmonary blood vessels. It would seem that the facts recorded in the foregoing sections

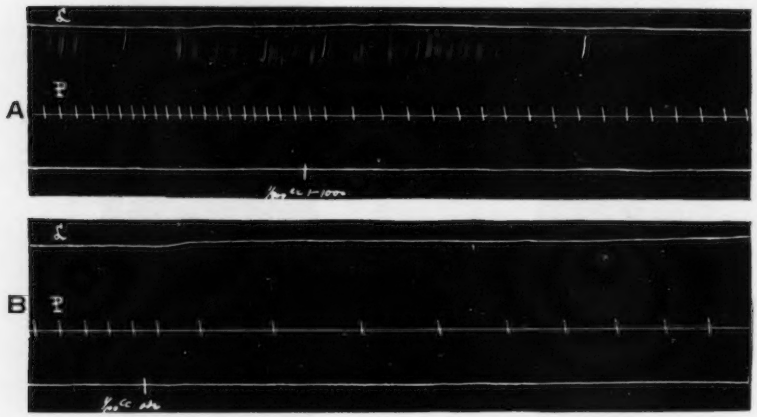

Fig. 6. Records of the intrapulmonic pressure, *L*, and venous outflow of the right lung of the frog in drops of frog blood-Ringer's mixture (mostly Ringer's), *P*, with lowermost line indicating by signal magnet the injection of the drug, adrenalin chloride. Perfusion of the right lung by way of the left aortic arch, all other blood vessels distal to the right pulmonary artery (including the bulbus aortae) having been tied off. The perfusion mixture returning from the lung was collected by a cannula tied into the auricle through the sinus. All injections made by hypodermic needle at cannula of inflow into the lung. Showing in A slight vasoconstrictor action following the perfusion of the lung with $\frac{1}{200}$ cc. of 1:1000 adrenalin solution; in B, pronounced vasoconstrictor action on doubling the dose.

are sufficient proof that the blood vessels of the frog's lung are supplied with vasomotor nerves through the vagus system. Nevertheless a number of experiments was made with the action of epinephrin on the perfused lung, partly because of the contradictory results obtained from epinephrin by previous investigators in the case of the mammalian lung, and partly because of the prevalent view that epinephrin is specific for sympathetic nerve endings, and in case of the pulmonary blood vessels

of the frog the vasomotor nerves most probably belong to the vagus system, not to the sympathetic. Will epinephrin act on the vagus nerve endings?

Typical records showing the effect of epinephrin on the tonus of the pulmonary blood vessels of the frog are reproduced in figures 5 and 6. Primary vasodilatation from small doses of epinephrin is obtained only in very fresh preparations. The prolonged vasoconstrictor action from larger doses of the drug can be demonstrated in a perfused preparation over a somewhat longer period. But the blood vessels of the perfused lung soon (10 to 20 minutes) cease to react to epinephrin in any concentration. But there is no question of the vasoconstrictor action of epinephrin in moderately large doses in the fresh preparation.

In comparing our results on the perfused lung with those of previous investigators on perfusion of the frog's hind legs, it seems that the pulmonary vessels are less sensitive to epinephrin than are the blood vessels of the extremities, a conclusion previously arrived at by Plumier (21) during similar perfusion experiments of the mammalian lung. We believe this difference is only apparent and due to the rapid deterioration of the lung tissues under perfusion, as compared to the extremities. In any event, epinephrin has the same action on the lung arteries as on the arteries of the systemic circulation, despite the fact that the pulmonary vasomotor nerves belong to the vagus system.

The action of histamine. We have reported elsewhere that intravenous injection of histamine in the frog may cause lung dilatation or lung contraction, depending on the concentration of the drug and the physiological condition of the local nervous system in the lung. If the perfused lung is in fresh condition the pulmonary blood vessels are more sensitive to histamine than the lung motor tissues, that is, concentrations of histamine that have little or no action on the lung tonus cause a marked and prolonged contraction of the lung blood vessels (fig. 7). It should also be noted that the perfused lung very soon fails to respond to histamine in any concentration.

4. The paralysis of the pulmonary vasomotor nervous mechanism by atropine. The discovery that atropine abolishes the vasomotor action of the vagus nerve on the lung arteries, made early in the work, created at first a very perplexing situation. So far as we know, atropine does not paralyze vasomotor nerves in any other organ in any species of animals investigated. We observed the fact in attempting to eliminate the cardiac factor (on vagus stimulation) by atropine, following the lead of nearly all previous workers on the mammalian lung. Prepara-

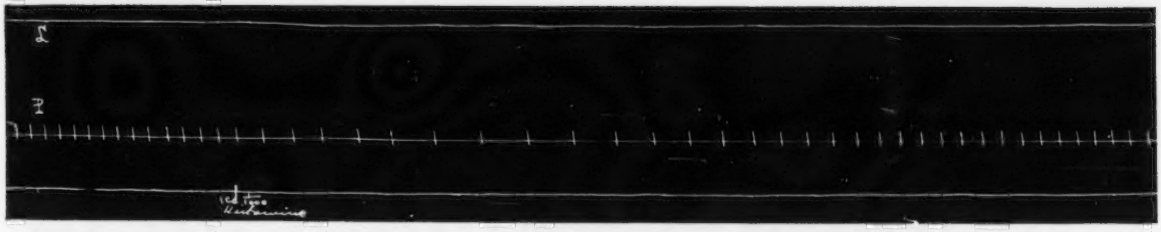

Fig. 7. Record of the intrapulmonic pressure of the right lung of a frog *L*, and rate of perfusion in drops, *P*, issuing from the same lung before and after the perfusion of the lung with 1 mgm. of histamine as indicated by signal magnet on lowermost line of the tracing.

Frog decerebrated and spinal cord transected and pithed below the medulla. Cannula in tip of right lung, glottis closed with hemostat. Perfusion through left aortic arch, all blood vessels except right pulmonary artery ligated off. Bulbus aortae ligated off. Return flow from lung through cannula tied in auricle of heart.

Showing marked vasoconstriction of the pulmonary vessels with subsequent return following perfusion of the lung with histamine.

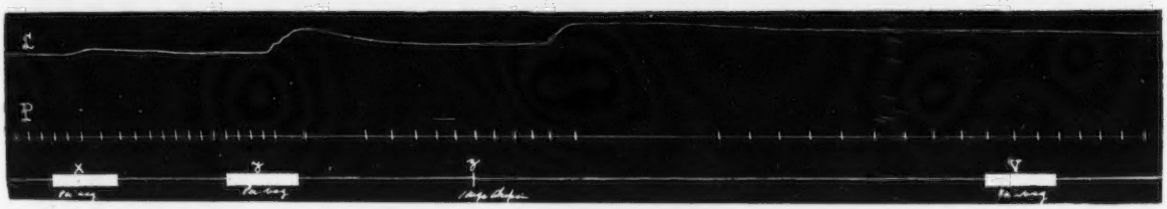

Fig. 8. Record of the intrapulmonic pressure, *L*, of the right lung of a frog with signal magnet record, *P*, of drops of blood-Ringer mixture issuing from the right lung.

Animal prepared as described in text and in legend of figure 7.

x = Stimulation of vagus with weak tetanizing after nicotinization of the lung.

y = Same, using stronger current.

z = Perfusion of the lung with 1 mgm. atropine sulphate.

V = Electrical stimulation of the peripheral end of the vagus after atropine. Showing that the vasomotor and lung motor action of the vagus is abolished by atropine.

tions that showed marked vasoconstriction in the lung on vagus stimulation failed to show this effect after sufficient atropinization to paralyze the cardiac vagus. Now, as atropine also abolishes the lung motor fibers in the vagi, the suggestion was obvious that our apparent vasomotor action in the lung is in some way due to change in the lung tonus. This unexpected action of atropine called for special scrutiny of our experiments, and devising of additional tests to place the matter beyond controversy. We believe this has been done in the following way: When atropine is injected into the circulation the lung motor and the vasomotor fibers are paralyzed practically at the same rate (fig. 8), but

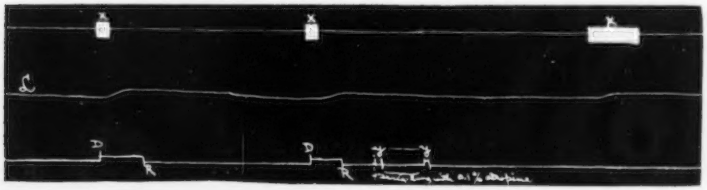

Fig. 9. Record of the intrapulmonic pressure, L, of the right lung of a frog with signal magnet record indicating at D complete disappearance and at R complete reappearance of the large superficial pulmonary vessels on electrical stimulation of the right peripheral vagus at x before and after irrigation of the lung (y–y) with several drops of 0.1 per cent solution of atropine sulphate.

Frog decerebrated, spinal cord transected below medulla and pithed. Cannula in tip of right lung. Cardio-inhibitory effects of vagus abolished with nicotine introduced into the pericardial sac. At x, electrical stimulation of the peripheral end of the vagus.

Showing paralyzing action of atropine on the vasomotor fibers before the lung motor fibers and indicating furthermore that the contraction of the lung musculature itself is not responsible for the complete vasoconstriction seen before the application of atropine.

when a few drops of dilute atropine solution are painted directly on the pulmonary arteries, the vasomotor nerves fail before the lung motor nerves. A record illustrating this selective action is reproduced in figure 9.

These results project two discordant facts into the foreground of the pharmacology and physiology of epinephrin and atropine, the latter paralyzing the pulmonary vasomotor nerves, and the former exhibiting the typical hemodynamic action on blood vessels that are innervated, not by the sympathetic but by the vagus. We are sure of the facts, no matter how divergent they are from the prevalent views of the chemotaxis of these drugs.

5. Vasomotor reflexes in the lungs. Having demonstrated the presence of vagal vasomotor nerves to the pulmonary arteries and their tonic activity, we naturally became interested in the reflex control of this mechanism. All our observations on this phase were made on preparations sufficiently curarized to eliminate the readily elicited cardio-inhibitory reflex, and direct inspection of the lung arteries had to be relied on to determine reflex effects. It needs scarcely to be pointed out that direct inspection can detect marked vasoconstriction or vasodilatation only. Slight vasomotor changes will escape detection.

In decerebrated and lightly curarized frogs marked reflex vasoconstriction can usually be produced in the lung by tetanization of the central end of the opposite vagus or the central end of the brachial nerve on either side. This probably occurs against a rise in general arterial pressure. Reflex vasodilatation in the lung could never be detected by our method of observation. Theoretically, it should occur even in the absence of vasodilator nerves, through central inhibition of the tonus of the vasoconstrictors.

We have been impressed by the difficulty in securing reflex vasomotor effects in the frog's lungs in comparison with the ease that lung motor, cardiac, and skeletal reflexes are obtained in this species from all afferent nerves. But this difference may be only apparent or due to the failure of noting slight changes in the caliber of the pulmonary arteries by direct inspection.

RESULTS ON THE TURTLE

1. Influence on the pulmonary blood vessels of section of the vagi and the sympathetic nerves. In several preparations the pulmonary arteries were found markedly constricted after isolation of the lungs by our method of preparation. This might be due to mechanical injury to the lung arteries in the process of lung isolation. We have previously called attention to the fact that in the turtle mechanical handling of even a large artery like the carotid may cause extreme tonic constriction of the artery lasting for 10 to 30 minutes. The pulmonary arteries behave in the same manner. It is therefore necessary to exercise great care in isolating the lungs so as not to subject the lung arteries to rough mechanical handling. The type of tonic arterial constriction due to direct mechanical stimulation is always local or confined to the region of the artery subject to direct handling. This fact renders it a priori improbable that a tonic vasoconstriction

extending uniformly over the entire lung is due to direct mechanical injury. In such preparations *section of the vagus nerve in the neck leads to a prompt and lasting vasodilatation in the lung of the same side.* This is not due to improvement in the heart and a higher arterial pressure. Were this the cause, the vasodilatation should occur in both lungs. The fact is that it is always confined to the lung on the side of the vagus section. It also occurs in preparations in which the vagus section does not augment the heart beat or raise the arterial pressure.

Section of the cervical sympathetic had in no case any effect on the caliber of the pulmonary vessels. Moreover, section of the vagus close to its exit from the skull has the same effect as section of the nerve further peripherally after the cervical sympathetic nerve has joined the vagus trunk. These experiments with direct observation have

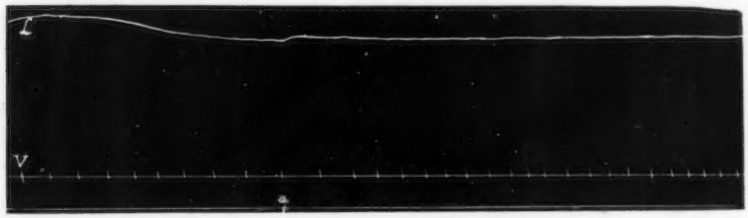

Fig. 10. Perfusion of left lung (in situ) of a decerebrated and curarized turtle, fixed dorsal side down and plastron removed. Left vagus intact; perfusion fluid, Ringer's defibrinated turtle blood. *L*, record of intrapulmonic pressure; *V*, rate of venous outflow (drops). *a*, section of left vagus in the neck. Showing vasodilatation in the lungs following section of the vagus.

been corroborated on lungs perfused in situ, but with the vagus nerve intact. A typical record showing about 75 per cent increase in the rate of blood flow through the lung following section of the vagus nerve is reproduced in figure 10.

In most of our experiments records were taken of the intrapulmonic pressure. The section of the vagus nerve produces no changes in the lung tonus or lung contraction that can account for the permanent dilatation of the pulmonary vessels. We must therefore conclude that the pulmonary arteries in the turtle are provided with vasomotor nerves through the vagi, and that this vasomotor mechanism is, at least in some animals, in tonic activity. In our most favorable preparations the hyperemia of the lung following section of the vagus nerve is as marked as the hyperemia of the ear following section of the cervical sympathetic nerve.

But why should we not be able to demonstrate this pulmonary-vasomotor tonus in all of our preparations? In many animals the section of the vagus nerve did not appreciably alter the caliber of the pulmonary arteries. This was particularly true in animals in poor condition from long captivity or other causes. We were probably also dealing with the factor of traumatic and nervous shock, unavoidable from the method of preparing the animals. The temperature of the animal may be a factor as it is in the frog. We have only worked on turtles taken from cold water. In no instance did we warm up the turtles as we did the frogs before preparing them for experimentation.

2. Pulmonary vasomotor rhythm. In some of our preparations periodic alterations in the caliber of the pulmonary arteries were noted similar to those described for the frog. In the turtle this was seen both in the intact and the denervated lung. The mechanism of this rhythm is therefore partly peripheral. The periodic changes in the caliber of the arteries is so marked that there is no mistaking it even with direct inspection. This peripheral vasomotor rhythm is independent of the tonus of the lung motor tissues (fig. 11). This vasomotor rhythm was seen more frequently in the perfused lung than in lungs with the normal circulation intact. Similar periodic changes in the caliber of the pulmonary vessels of the mammalian lung were noted by Cavazanni (7).

3. The influence on the pulmonary blood vessels of stimulation of the vagus and the sympathetic nerves. This phase of the question was investigated on isolated lung perfused in situ or after excision from the body, as well as by direct inspection of the lung with the circulation intact. In every case controls were run on the lung contraction induced by the vagus stimulation. Typical records illustrating the results obtained are reproduced in figures 12 to 15. Weak tetanization of the peripheral vagus in a perfused lung may cause vasodilatation (fig. 12). But in most of the preparations all strengths of vagus tetanization that had any effect on the pulmonary circulation at all, caused vasoconstriction (figs. 13 and 14). The strength of the tetanizing current required to influence the pulmonary vessels when applied to the peripheral vagus causes at the same time contraction of the lung musculature. The lung, excised or in situ, perfused with a mixture of Ringer plus defibrinated turtle blood deteriorates gradually, though not as rapidly as the perfused lung of the frog. In a perfused turtle lung gradually deteriorating the pulmonary vasomotor mechanism fails long before the failure of the lung motor mechanism. In fact some preparations showing good vasoconstriction of the lungs on vagus stimulation when the

Fig. 11. Perfusion of excised lung of the turtle with Ringer + defibrinated turtle blood. *L*, record of intrapulmonic pressure (lung tonus). *V*, rate of venous outflow in drops. Showing peripheral spontaneous variations in tonus of the pulmonary blood vessels. These tonus variations were evident on direct inspection of the lung.

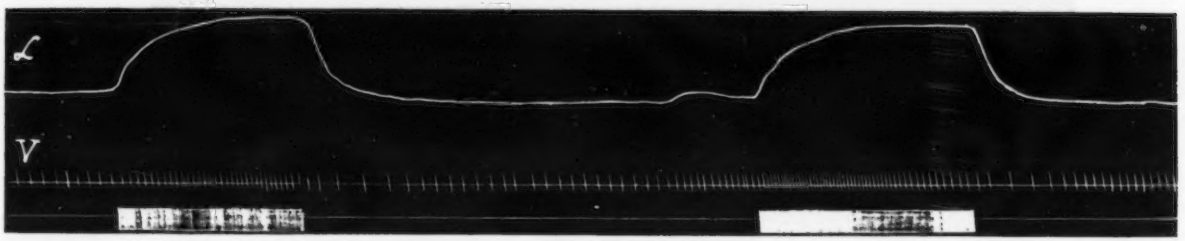

Fig. 12. Record of lung tonus and lung perfusion in the turtle. Animal decerebrated, fixed dorsal side down, plastron removed, and viscera lifted so as not to press on lung. Perfusion of Ringer + defibrinated turtle blood through left pulmonary artery. *V*, rate of pulmonary venous outflow (drops). Signal, tetanization of peripheral end of left vagus, showing vasodilatation parallel with the lung tetanus.

Fig. 13. Excised lung of turtle, perfused with Ringer's defibrinated turtle blood. Bronchus, ligated, cannula in tip of lung. *L*, record of lung tonus. *V*, rate of venous outflow in drops. Signal, tetanization of lung vagus. *a*, with secondary coil at 15 cm.; *b* and *c*, at 10 cm. Showing lower excitability and more rapid fatigue of the pulmonary vasomotor mechanism than of the motor mechanism of the lung itself.

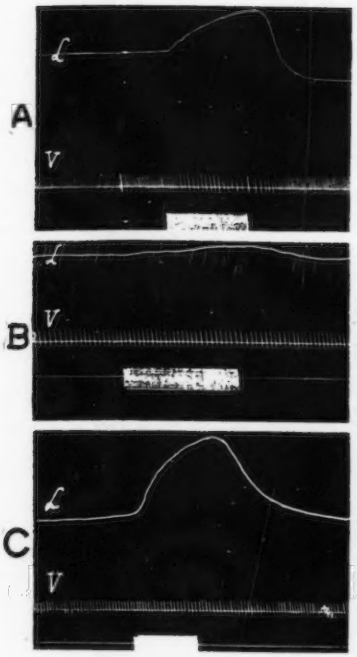

Fig. 14

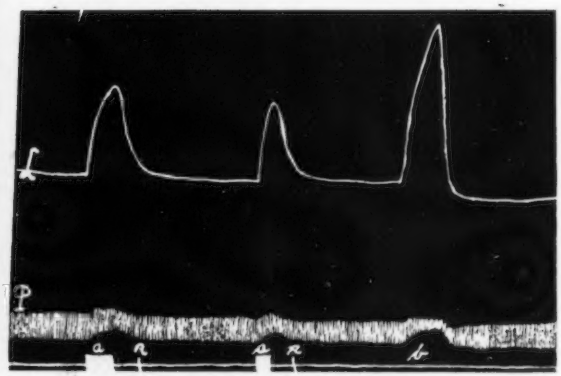

Fig. 15

Fig. 14. Excised lungs of the turtle, perfused with Ringer's solution + defibrinated turtle blood. Bronchus ligated, cannula in tip of lung. *L*, record of lung tonus. *V*, rate of venous outflow in drops. Signal, tetanization of the lung vagus. A and B, records from left lung in fresh condition, ½ mgm. atropin sulphate injected into perfusion solution near the pulmonary artery between records A and B. C, record from right lung of the same turtle 26 hours later. Showing pulmonary vasoconstrictor action of the vagus *A*, paralysis of this action by atropin *B*, and more rapid failure of the vasomotor mechanism than of the lung motor mechanism as the lung is placed in non-physiological conditions, *C*.

Fig. 15. Records of the intrapulmonic pressure, *L*, and arterial blood pressure, *P*, (right pulmonary artery) in the turtle. Animal decerebrated and curarized. Left lung isolated from the dorsal side, the bronchus ligated, and the cannula for recording the intrapulmonic pressure fixed in the tip of the lung. Left vagus sectioned in the neck, the cardiac and gastric branches of the vagus sectioned, leaving only the pulmonary branches intact.

a, Stimulation of the peripheral end of the left vagus with a weak tetanizing current. The end of tetanization indicates the complete disappearance of the pulse in the arteries of the left lung; *r*, reappearance of the pulse in the left lung; *b*, raising the intrapulmonic pressure (left lung) by air inflation above that due to the lung contraction following tetanization of the vagus. This artificial raising of the intrapulmonic pressure did not obliterate the pulse in the lung arteries.

circulation was left intact, failed to respond with vasomotor action when artificial perfusion had been established. Even in our best preparations the vagus acts on the pulmonary arteries only during the first 10 to 15 minutes of the perfusion, while the vagus lung motor mechanism may persist for hours in the perfused lung. When the peripheral vagus (the cardiac fibers being sectioned) is tetanized with the lung in situ and the circulation intact, there is produced so marked a vasoconstriction in the lung that the pulse disappears even in the large branches of the pulmonary arteries (fig. 15), but the constriction is rarely great enough to completely obliterate the lumen of these arteries, as is the case in the frog's lung.

We have been unable to influence the lung blood vessels by the stimulation of the cervical sympathetic nerve. We have stimulated this nerve intact and with the vagus intact; also the peripheral end with the vagus intact, and the central end both with sectioned and intact vagus, all with equally negative results no matter whether the lungs were isolated from the ventral or the dorsal side. It would therefore seem that in the turtle, as in the frog, the pulmonary vasomotor nerves belong to the vagus system.

We have stated, and accompanying tracings show the fact, that in all cases where tetanization of the peripheral vagus causes pulmonary vasomotor action there is also contraction of the lung. Is there not a possibility that the vasomotor changes are mechanical effects due to the contraction of the lung musculature? This question can be answered in the negative, for the following reasons:

a. In preparations in which the vasomotor nerves have failed owing to poor physiological condition the vagus stimulation causes lung contraction, but no change in the caliber of the blood vessels. Hence lung contractions do not necessarily produce demonstable changes in the caliber of the blood vessels or the rate of the blood flow (figs. 13, 14).

b. Increasing the intrapulmonic pressure artificially (by inflation) even above that caused by the active lung contraction does not obliterate the lung arterial pulse, at least not to the same extent that it is done by the vagus stimulation (fig. 15). Of course, raising the intrapulmonic pressure by inflation produces different mechanical conditions on the lung blood vessels than those produced by the lung contractions. And it is possible to raise the intrapulmonic pressure by inflation to such a degree that the arterial pulse of the intact lung is weakened, and the flow of blood through the perfused lung retarded.

c. Certain drugs (epinephrin, histamine, pituitrin) act both on the lung motor and the lung vasomotor mechanism, but the actions on these two mechanisms do not run parallel. For example, epinephrin, causes vasodilatation or constriction parallel with or without relaxation of the lung musculature. Histamine causes vasoconstriction and lung contraction, but the vasoconstriction persists longer than the lung contraction.

d. On theoretical ground it seems possible that the active lung contraction by pressure on lung capillaries and veins may temporarily augment the venous outflow, and thus give the effects of vasodilatation. We have tested this possibility by occluding the lung artery prior to the tetanization of the vagus in the perfused lung. Under these conditions the lung contraction caused by the tetanization of the vagus nerve does not start up a temporary venous outflow from the lung. It seems therefore clear that tracings like that reproduced in figure 12 cannot be due to this mechanical factor.

It is true that the relation of the main lung arteries to the lung musculature is not as simple in the turtle as in the frog. In the frog the main branches of the lung arteries run on the surface of the lung; in the turtle these arteries, at least in part of their course, pass through the body of the lung, and may therefore be more directly influenced by the tonus of the lung musculature. But the facts enumerated (*a*, *b*, *c*, *d*) show conclusively that the vasomotor changes in the lung following tetanization of the peripheral vagus are direct vasomotor effects and not mechanical products of the changes in tonus of the lung musculature.

4. Action of epinephrin, histamine and pituitrin on the pulmonary blood vessels. The action of these drugs was studied both by introduction into the general circulation and by addition to the perfusion mixture of the isolated lung. The latter method gives the most satisfactory data as it eliminates the change in general arterial pressure and the uncertainties of direct inspection.

If the perfused lung is in good condition the weakest concentration of epinephrin that affects the lung blood vessels at all causes vasodilatation (figs. 16, 17). If the lung blood vessels are in marked tonus the vasodilatation caused by the minimal quantities of epinephrin may increase the rate of the blood flow through the lung several hundred per cent. Larger quantities of the drug have the opposite effect or a vasoconstrictor action, which in fresh preparations may last 10 to 15 minutes (fig. 18). The older and poorer the preparation the feebler and shorter the

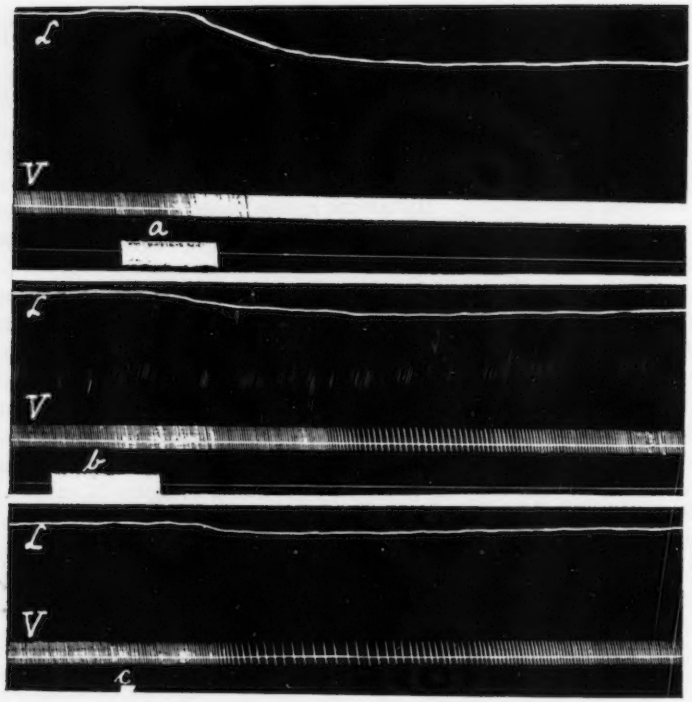

Fig. 16. Excised lung of the turtle. Perfused through pulmonary artery with Ringer solution + a small quantity of defibrinated turtle blood. Bronchus ligated, cannula for recording intrapulmonic pressure in tip of lung. *L*, record of lung tonus. *V*, venous outflow in drops. Addition of adrenalin to the perfusion solution close to the pulmonary artery, *a*, 0.1 cc.; *b*, 0.2 cc.; *c*, 0.5 cc. adrenalin (1:1000). Showing inhibition of lung tonus, and vasodilator and constrictor action of adrenalin on the pulmonary vessels.

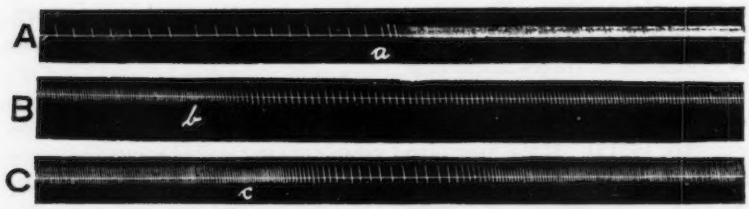

Fig. 17. Perfusion of isolated lung of turtle with Ringer + defibrinated turtle blood. *A*, lung vessels in strong tonus. *B* and *C*, lung vessels dilated. *a*, *b*, *c*, injection of $\frac{1}{20}$ cc. adrenalin (1:1000) into perfusion fluid into pulmonary artery. Showing opposite vasomotor action of the same concentration of adrenalin depending on the tonus of the vessels.

vasomotor response to epinephrin, and lungs perfused for 30 to 60 minutes with a Ringer-blood mixture and still giving good lung contractions in response to the tetanization of the vagus may fail to show any vasomotor effect even when enormous quantities (1 cc. 1:1000) of the drug are added to the perfusion mixture.

If the excised and perfused lung is in a state of marked tonus epinephrin in quantities that induce vasomotor action causes at the same time inhibition of the lung tonus (fig. 16).

On the basis of the prevailing theory of epinephrin vasomotor action, the above results show that the pulmonary blood vessels of the turtle are provided both with dilator and constrictor nerves. We have already adduced proofs of the presence of these nervous mechanisms independent of the epinephrin data, but the latter are interesting and may be interpreted as confirmatory. In the turtle as in the frog, epinephrin appears to exert its typical hemodynamic action on nerves that belong to the vagus, not to the sympathetic system.

Histamine as well as pituitrin causes pulmonary vasoconstriction and lung contraction (figs. 19, 20). The actions of these drugs on the two motor systems do not run parallel. In the case of the excised and perfused lung, at least, the blood vessels are more sensitive than is the lung musculature.

5. The action of atropine on the pulmonary vasomotor mechanism. Atropine in doses that paralyze the cardiac and the pulmonary vagi abolishes at the same time the vagus action on the lung blood vessels, just as in the frog. In fact, this drug may completely abolish the vasomotor lung action before the paralysis of the lung motor action (fig. 14 *C*).

The comments made in connection with this phenomenon in the frog need not be repeated here.

6. Lung vasomotor reflexes. Our attempts to secure vasomotor reflexes into the lung were not extensive. As in the frog, the vasomotor reflexes are not readily elicited, at least in animals prepared by our method. This may be due to a condition of prolonged "shock" of the pulmonary vasomotor center in the medulla or to the complexity of the peripheral nerve mechanism. It may be noted that motor reflexes into the stomach are, if anything, difficult to produce.

With the lung exposed and left with the circulation intact pulmonary vasomotor reflexes must be judged by direct inspection of the lung blood vessels. Slight changes in the caliber of the vessels cannot be detected by direct inspection. Perfusion of the lung in situ, with

the pulmonary vagus intact, permits more accurate records, but this method has the disadvantage of rapid failure of the vasomotor nerves. In figure 21 typical tracings secured by this method are reproduced, showing slight vasomotor action (dilator and constrictor) on stimulation of the posterior nares, and the central end of the sciatic nerve. Our few experiments on this phase indicate at least that pulmonary

Fig. 18. Record of perfusion of the excised lung of the turtle with Ringer + defibrinated turtle blood. Signal, introduction of $\frac{1}{20}$ cc. 1:1000 adrenalin in perfusion fluid near the pulmonary artery. Showing intense and prolonged vasoconstrictor action of the drug.

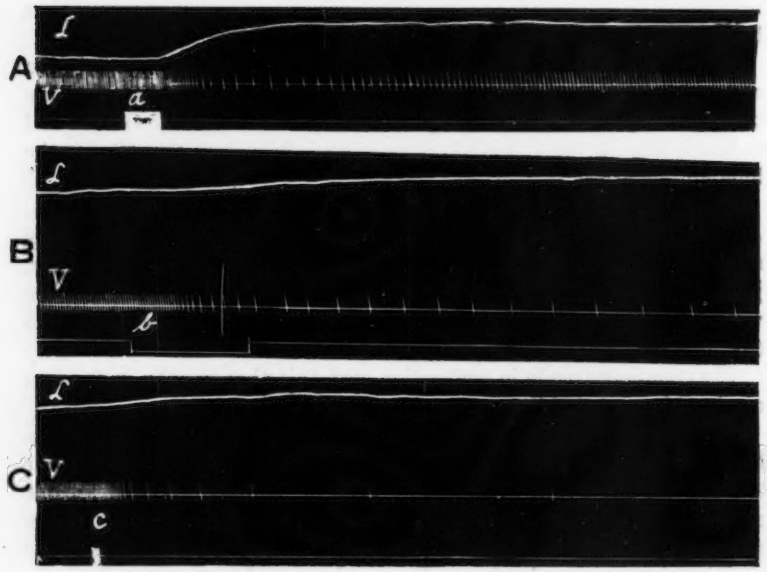

Fig. 19. Transfusion of the excised lung of the turtle with Ringer + defibrinated turtle blood. *L*, record of lung tonus (intrapulmonic pressure). *V*, rate of venous outflow (drops). Addition of histamine chloride to perfusion fluid near pulmonary artery, *a*, $\frac{4}{10}$ cc.; *b*, $\frac{1}{10}$; *c*, $\frac{3}{10}$ cc. 1:1000. Showing histamine contracture of the pulmonary blood vessels independent of the histamine tetany of the lung musculature.

vasomotor reflexes are much more difficult to elicit than the motor reflexes into the lung musculature.

7. The peripheral lung tonus and the secondary action of the vagus on this tonus. In our previous studies on lung automatism in the turtle,

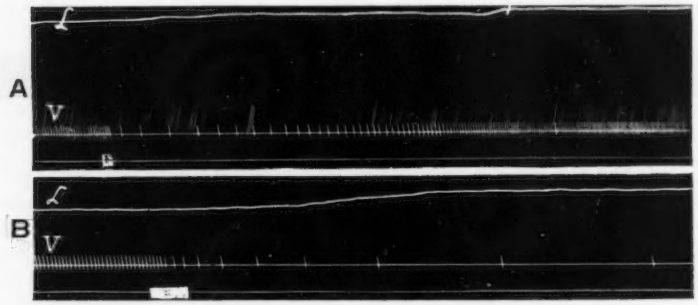

Fig. 20. Record of perfusion of the excised lung of the turtle with Ringer + defibrinated turtle blood. *L*, record of lung tonus (intrapulmonic pressure). *V*, rate of venous outflow (drops). A, signal, addition of ½ cc.; B, signal, 1 cc. pituitrin to perfusion fluid near pulmonary artery. Showing pulmonary vasoconstrictor action of pituitrin independent of the contraction of the lung musculature.

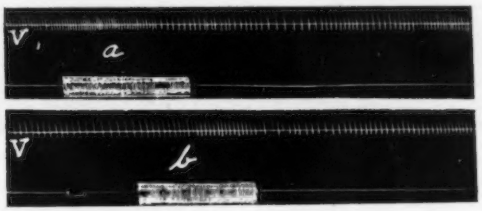

Fig. 21. Perfusion of the turtle lung (right) in situ. Animal decerebrated, curarized, and fixed dorsal side down. Anterior third of plastron removed to permit access to pulmonary vessels. Right vagus intact. Perfusion fluid, Ringer + defibrinated turtle blood. *V*, rate of venous outflow (drops). *a*, tetanization of central end of sciatic nerve; *b*, mechanical stimulation of the posterior nares. Indicating pulmonary vasomotor reflexes. (Record of the intrapulmonic pressure not obtained owing to mucus in the bronchus.)

we secured little or no evidence of the existence of inhibitory nerves to the lung motor tissues, and very little indication of a peripheral tonus independent of the motor fibers of the vagi and the activity of the cen-

tral nervous system. To be sure, a feeble lung rhythm was sometimes seen in the lungs after section of the vagi nerves.

In the present series we have frequently noticed a gradual increase in the lung tonus during perfusion with the Ringer-blood mixture, both of the excised lung and of the lung left in situ, and the vagus intact. On this tonus contraction there is always superimposed a feeble but

Fig. 22. Record of lung (left) tonus in a decerebrated and curarized turtle. Animal fixed dorsal side down. Platron removed. Left bronchus ligated. Cannula in tip of lung. Viscera removed from ventral surface of lung. Lung perfused through the pulmonary artery with Ringer + defibrinated turtle blood. *x*, section of left vagus in the neck. Showing a gradual increase in the lung tonus with indications of a peripheral tonus rhythm after section of the vagus. The intervals between the tracing sections A, B, C and D = about 10 minutes.

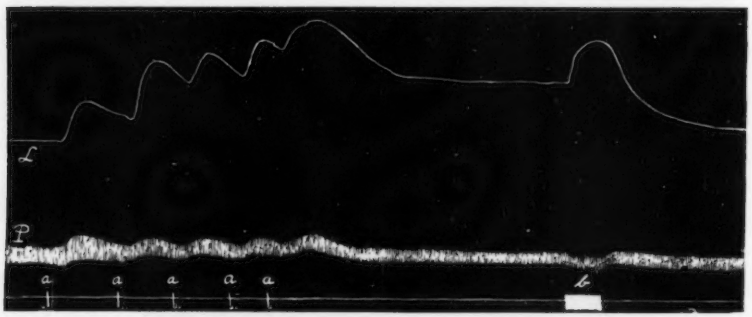

Fig. 23. Records of the blood pressure (right pulmonary artery) and left lung, *L*, tonus in a decerebrated and curarized turtle. Left lung isolated, except for pulmonary blood vessels and vagus. *a*, repeated injections into the jugular vein of $\frac{1}{20}$ cc. 1:100 histamine chloride; *b*, tetanization of left peripheral vagus. Showing inhibition of the histamine tetanus following the primary lung contraction on vagus stimulation.

distinct rhythm of varying rate. In the preparations where we perfused the lung *in situ* and with the vagus intact, it was noted at times that *the tonus rise began on section of the vagus nerve* (fig. 22). This seems to indicate that there are some inhibitory fibers to the lung motor tissues in the vagi nerves. The fact that epinephrin inhibits the tonus in the turtle lung points in the same direction, if one may accept the theory of exclusive nerve ending action of this drug.

We also wish to record another fact observed in this series that may be interpreted on this basis. If the perfused lung, excised or in situ, is in a state of considerable tonus, stimulation of the vagus still causes further contraction of the lung musculature, but following the stimulation the relaxation of the lung musculature does not stop at the tonus level existing prior to the stimulation but drops much lower, and this secondary tonus inhibition lasts for some time. This phenomenon occurs whether the lung tonus is spontaneous or induced by such drugs as histamine (fig. 14 *A*, fig. 23). We recognize that this fact is capable of several interpretations, such as depressor action of fatigue metabolites, etc. It may also be due to the action of inhibitory fibers in the vagus nerve outlasting the more direct and powerful action of the motor fibers.

SUMMARY AND CONCLUSIONS

Frogs: 1. Section of the vago-sympathetic nerves leads to dilatation of the lung arteries on the same side, parallel with the development of hypertonus of the lungs. This tonic constrictor action of the vago-sympathetic nerve on the lung arteries appears to be due to vagus fibers, as section of the sympathetic nerve by itself is without effect on the lung.

2. Stimulation of the peripheral end of the vago-sympathetic nerve induces contraction of the lung arteries on the same side. In good preparations this contraction is maximal. The pulmonary vasoconstriction is independent of the vagus action on the heart (elimination of cardiac vagus by curare, nicotine, direct perfusion of the lung, and excision of the heart) and on the lung motor tissues, as it may occur parallel with lung contraction, lung dilatation or no change in lung tonus. Stimulation of the peripheral end of the cervical sympathetic nerve has no effect on the tonus of the pulmonary arteries. Hence the vasoconstrictor fibers appear to belong to the vagus system.

3. In the lung perfused with a mixture of frog's blood and Ringer's solution minimal doses of epinephrin have a vasodilator action; larger doses, a vasoconstrictor action. The perfused lung deteriorates rapidly and these vascular responses to epinephrin occur only in very fresh preparations.

4. Histamine and pituitrin cause vasoconstriction in the lungs. The vascular motor tissues are more sensitive to these drugs than the lung motor tissues.

5. Atropine in doses that paralyze the cardiac vagus and the lung motor fibers in the vagus abolishes also the vasomotor action of the vagus on the pulmonary blood vessels.

6. Pulmonary vasoconstrictor reflexes have been obtained from the central vagus and the brachial nerves.

Turtles: 1. Section of the vagus causes dilatation of the pulmonary arteries on the same side. This is not due to cardiac and arterial blood pressure changes, because it occurs only in the lung on the side of the vagus section, and it occurs in the lung perfused *in situ*. Section of the cervical sympathetic nerve has no action on the tonus of the pulmonary blood vessels. The tonic vasoconstrictor action in the lungs appears therefore to be due to vagus fibers.

2. Periodic variations in the tonus of the pulmonary arteries may occur in the intact lung. These tonus variations may disappear on section of the vagus on the same side. A vasomotor rhythm of strictly peripheral origin has also been observed in the denervated lung, particularly under perfusion.

3. Stimulation of the peripheral end of the vagus nerve causes vasoconstriction in the lung of the same side. In a few preparations weak tetanization of the vagus caused vasodilatation in the lung. Stimulation of the cervical sympathetic nerve has no influence on the tonus of the pulmonary blood vessels.

The pulmonary vasomotor effects of tetanization of the vagus are independent of cardiac and arterial pressure factors (section of the cardiac vagus, direct perfusion of the lung), and are at least partly independent of the lung contraction induced by the stimulation.

4. In the lung perfused with a mixture of turtle's blood and Ringer's solution minimal doses of epinephrin cause pulmonary vasodilatation; larger doses, pulmonary vasoconstriction. The perfused lung deteriorates gradually, the vagus vasomotor action and the hemodynamic action of epinephrin disappear long before the lung motor action of the vagus.

5. The pulmonary vasomotor action of the vagus is abolished by doses of atropine that paralyze the cardiac and the lung motor vagi fibers.

6. Pulmonary vasoconstrictor reflexes have been observed from electrical stimulation of the central sciatic nerve and mechanical irritation of the posterior nares.

7. Histamine and pituitrin cause contraction of the pulmonary blood vessels. This action is independent of the contraction of the lung musculature induced by these drugs.

General: 1. We have been unable to obtain any pulmonary vasomotor action from the cervical sympathetic nerves in these animals. These are merely negative results, and we do not attach positive significance to them at present.

2. On the basis of the prevailing theory of epinephrin action, the pulmonary vasodilatation induced by minimal doses of epinephrin indicates vasodilator nerves to the lungs. In the turtle these vasodilator fibers appear to be included in the vagus; in the frog our work does not disclose the course of the vasodilator nerves.

3. Both in the frog and in the turtle atropine in pharmacological doses paralyzes the pulmonary vasomotor nerve mechanism. So far as we know this is the first clear demonstration of vasomotor paralysis by this drug. This fact appears to render questionable the work of previous investigators on the subject of pulmonary vasomotor control in the mammal where atropine was used to eliminate the cardiac factor.

4. It would seem that the pulmonary vasomotor reflexes (central and peripheral) must be taken into account in lung physiology and pathology. Drugs like epinephrin, histamine, atropine and pituitrin act parallel but independently on the vasomotor and lung motor mechanism.

5. The pulmonary vasomotor nervous mechanism is more readily fatigued and more quickly paralyzed by untoward physiological conditions than any other vasomotor mechanism known to us in the mammal. This may indicate a complex system of neurones on the pulmonary vasomotor pathway. This fact serves, at least in part, to explain past failures in the research on vasomotor control in the lungs. We are apparently dealing with a mechanism having the delicate physiological balance of complex reflexes. The animal must be in good condition and the lung fresh, particularly if perfusion methods are used. Comparisons by the perfusion method may not be made with the lung and the skeletal muscles (hind legs). The lungs appear to be as delicate organs as some glands (e.g., the pancreas). For the lung of the frog and the turtle Ringer's solution is a poison, not a physiological solution, as it is for the skeletal muscles and motor nerves. The delicacy of the mechanism may also explain the apparent difficulty in inducing pulmonary vasomotor reflexes under our experimental conditions.

6. Our evidence goes to show that the pulmonary vasoconstrictor nerves belong to the vagus system, that is, they are not nerve fibers that join the vagus from sympathetic connections outside the skull. We have also seen that epinephrin causes vasoconstriction in these

lungs. It is idle to speculate whether these facts demand a revision of the theory of epinephrin hemodynamics or a further analysis (anatomical and physiological) of the correlation of the vagus and the sympathetic systems.

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PARADOXICAL PUPIL DILATATION FOLLOWING LESIONS OF THE AFFERENT PATHS

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In 1855 Budge (1) observed in a young rabbit, after section of the left cervical sympathetic nerve and of the branches distal (cepha'ad) to the right superior cervical ganglion, that the right was the smaller of the two pupils half an hour after the operation; whereas, 48 hours after the operation the right pupil was the larger of the two. In adult rabbits 24 hours after a similar operation he found the pupil larger on the side on which the post-ganglionic fibers had been cut. Following Budge many observers reported paradoxical effects in the pupil-dilator mechanism as the result of lesions in the course of the cervical sympathetic from the origin of its preganglionic fibers in the upper thoracic region of the spinal cord, to the ending of its post-ganglionic fibers in the region of the ciliary body and iris. The most noteworthy contributions on the subject came from Schiff (2), Kowalewsky (3), Braunstein (4), Lewandowsky (5), Langendorff (6), Anderson (7) and Meltzer and Auer (8).

Many observers noted the fact that after removal of one superior cervical ganglion, or section of one sympathetic nerve, the pupil on the side of operation under certain circumstances, e.g., asphyxia, excitement, injections of adrenalin, death, etc., became larger than its fellow. Because of the fact that the pupil on the side on which the efferent dilator path had been severed exhibited greater power of dilatation than its fellow, Langendorff (6) called the phenomenon paradoxical pupil dilatation, to the paradoxical phenomena already noted by other observers. Anderson (7), as the result of his investigations, added the paradoxical pupillary effects (constriction) consequent on section of the third cranial nerve or the equivalent of this, viz., removal of the ciliary ganglion or section of the short ciliary nerves. Elliott (9) found that paradoxical phenomena ensued in every organ supplied by the sympathetic system after section of the pre- or post-ganglionic fibers passing to that

organ. It should be noted that the paradoxical effects seen after lesions of the cervical sympathetic are not limited to the pupil but include widening of the *fissura palpebrarum*, withdrawal of the *membrana nictitans*, and bulging or *proptosis* of the eyeball. In the present studies attention was directed chiefly to alterations in the pupils and membranes. The width of the pupils, protrusion of the membranes, etc., were *estimated* where it is not expressly stated that actual measurements were taken.

None of the observers quoted, and none in the author's recollection, has made reference to the possibility of paradoxical effects consequent upon lesions of the afferent pathways, although it has long been known that stimulation of sensory nerves causes pupil dilatation. In the present studies after nerve section, etc., in cats, tests were made from time to time for paradoxical phenomena chiefly by injections of adrenalin into the jugular vein as first practised by Lewandowsky (5) and subsequently by Langley (10) and by Meltzer and Meltzer (11). Other methods of evoking paradoxical effects were occasionally employed such as asphyxiation, intravenous injections of pituitrin and of lactic acid, and finally instillations of adrenalin into the conjunctival sacs as practised by Meltzer and Auer (8). The condition of the pupils in death was carefully recorded as the death agony is a potent means of evoking paradoxical effects. When not otherwise stated adrenalin, etc., employed as an excitant of paradoxical effects, was administered intravenously. All operations were done under ether anesthesia. Before acceptance each animal was tested with adrenalin. The failure of conjunctival instillations of adrenalin to elicit frank paradoxical effects after sciatic section, etc., in instances in which paradoxical effects were readily elicited by adrenalin intravenously is of no significance since such instillations were often ineffective after cervical sympathetic section where adrenalin intravenously elicited paradoxical effects.

Section of one sciatic nerve. Numerous experiments were made in which one sciatic nerve was divided. In all instances paradoxical effects were readily evoked in the contralateral pupil by adrenalin. Description is confined to three animals with abstracted protocols.

In cat 1 well-marked paradoxical phenomena were evoked in the left eye 13 days after section of the right sciatic nerve. On the 2nd day after excision of the scar-area and suture of the divided nerve ends the paradoxical phenomena were still demonstrable in the left eye but had disappeared by the 4th day and were again demonstrable on the 13th day. On the 21st day after excision of the scar and suture

of the nerve ends the paradoxical phenomena had almost disappeared, being only manifest in the constriction period after adrenalin when the left pupil was larger than its fellow. On this day $1\frac{1}{2}$ inches of the distal segment of the divided nerve were removed and the gap bridged with catgut sutures. Twenty minutes later the paradoxical phenomena were about as immediately before the operation but were absent after that for 8 days and were demonstrable again 20 days after the operation. (Photograph 1.)

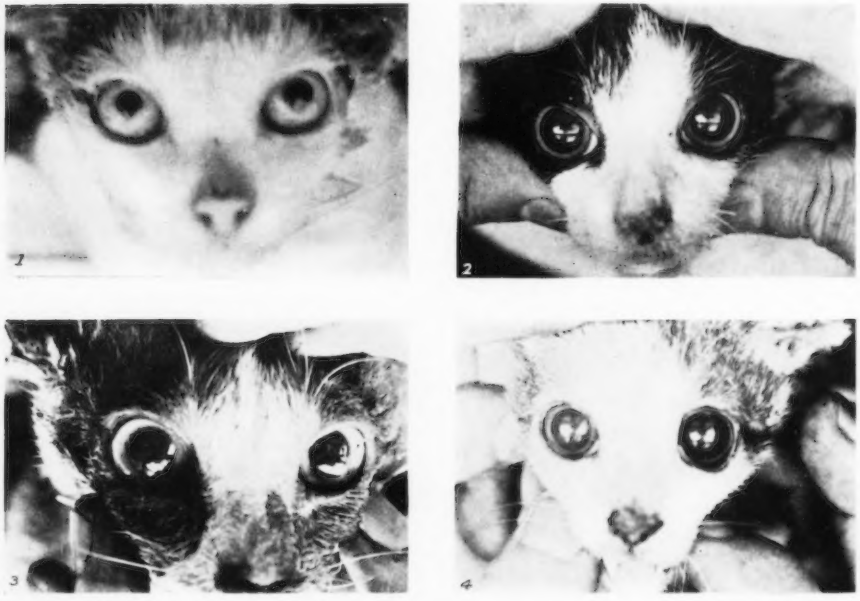

Fig. 1. Reappearance of paradoxical effects in the left eye after "freshening" the end of the proximal segment of the right sciatic nerve which had been sutured 41 days previously.

Fig. 2. Paradoxical effects in left eye on the 17th day after division of the right sciatic nerve and 7 minutes after section of both cervical sympathetic nerves.

Fig. 3. Reappearance of paradoxical effects in the right eye on 10th day after excision of the scar area in the left sciatic nerve which had been divided 18 days previously.

Fig. 4. Paradoxical effects in the left eye on the 12th day after injection of the right sciatic nerve with 60 per cent alcohol and 16 minutes after cord transection between roots Lii and iii.

On the 85th and 99th day (51st and 65th after excision of the distal segment of the divided sciatic, etc.), adrenalin failed to evoke paradoxical effects in the left eye. On the 99th day (65th after their insertion) the catgut strands were removed. On the 10th and 13th days after this last operation adrenalin failed to evoke appreciable paradoxical effects

Fig. 5. Paradoxical effects in the left eye evoked by ether anesthesia on the 37th day after section of posterior spinal roots Lvii to Sii on the right side.

Fig. 6. Paradoxical effects in the left eye evoked by adrenalin given intravenously on the 37th day after section of the posterior spinal nerve roots Lvii to Sii on the right side.

Fig. 7. Paradoxical effects evoked by adrenalin instilled into the conjunctival sac on the 37th day after section of the posterior nerve roots Lvii to Sii on the right side and 1 hour after transection of the cord between roots Lii and iii.

in the left eye. Evidently the removal of the catgut strands had little effect upon the paradoxical mechanism. Histological examination failed to show regenerating nerve fibers along the course of the catgut strands. One centimeter was now excised from the extremity of the proximal stump of the divided sciatic. On the 7th day after this last

operation adrenalin failed to evoke paradoxical effects. On the 9th day paradoxical effects were evoked but were easily exhausted since a lethal dose of lactic acid given a few minutes later failed to elicit them.

Protocol. Cat 1. Female, adult, non-pregnant, non-lactating

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
	a.m.		*mm.*	*mm.*	*mm.*	*mm.*
		July 12, 1919. Cut right sciatic nerve				
13th	9:44	Before adrenalin	5.0	4.0	2.0	2.0
	9:44½	Adrenalin ♍ii	8.0	11.0	0.0	0.0
		Paradoxical effects in left eye				
	10:30	Excised scar area and joined nerve ends with silk sutures				
26th		13th after excision of scar area				
		Paradoxical effects in left eye				
34th		21st after excision of scar area				
		Paradoxical phenomena waning in left eye				
		Excised 1½ inches of the distal segment and bridged space between the nerve ends with fine catgut after freshening end of proximal segment				
54th		20th after excision of distal segment, etc.				
	p.m.					
	2:03	Before adrenalin	2.5	2.5		
	2:03½	Adrenalin ♍ii	6.0	7.5	0.0	0.0
		Paradoxical effects in left eye: photograph 1				
99th		65th after excision of distal segment				
		Paradoxical effects absent in left eye				
	4:50	Removed catgut strands. No evidence of regenerating nerve fibers				
112th		13th after removal of catgut strands				
		Paradoxical effects absent in left eye				
		Excised 1 cm. from extremity of proximal segment				
123rd		9th after excision of 1 cm. from proximal segment				
	2:11½	Before adrenalin	7.0	5.0	2.0	3.0
	2:12	Adrenalin ♍ii	10.0	11.0	0.0	0.0
	2:12½	In constriction	8.0	9.0	1.0	2.5
		Trace of paradoxical effects in left eye				

In cat 2 slight paradoxical effects were elicited in the left eye 5 days after section of the right sciatic nerve. After excision of 1½ inches of the distal segment of the divided nerve paradoxical effects were elicited

Protocol. Cat 2. Young male about 4 months old

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		August 9, 1919. Cut right sciatic				
5th		Trace of paradoxical effects in left eye				
		Excised 1½ inches of distal segment				
17th		12th after excision of portion of distal segment				
	a.m.					
	9:05	Before adrenalin	3.5	4.0	3.0	3.0
	9:05½	Adrenalin ♏ii	8.0	9.0	3.0	3.0
		Paradoxical effects in left pupil but not in left membrane				
	9:36	Transected spinal cord between spinal roots Lii and iii				
	9:52	Before adrenalin	5.0	5.0	3.0	3.0
	9:52½	Adrenalin ♏ii	10.0	8.5	2.0	2.0
		No paradoxical effects in left eye				
	10:19	Before adrenalin	7.0	7.5	3.0	3.0
	10:19½	Adrenalin ♏ii	11.5	9.0	1.0	1.0
		No paradoxical effects in left eye				
	11:05	Transected spinal cord between roots Cv and vi				
	p.m.					
	12:08	Before adrenalin	2.5	2.5	1.0	1.0
	12:08½	Adrenalin ♏ii in 5 cc. of 5 per cent dextrose solution	10.0	11.0	1.0	1.0
		Paradoxical effects in left eye				
	12:41	Cut right and left sympathetic nerves in neck				
	12:46	Dextrose 5 cc. of 5 per cent solution intravenously; no effect on pupils				
	12:48	Adrenalin ♏ii in 5 cc. of 5 per cent dextrose solution	11.5	13.0	1.0	0.5
		Paradoxical effects in left eye: photograph 2				
	a.m.					
18th	2:30	Animal died				
	8:15	In death	8.5	9.0	6.0	5.5
		Trace of paradoxical effects in left eye: actual measurements				

only at times on the 3rd day (8th after primary nerve section) whereas on the 9th day (14th after the primary nerve section) paradoxical effects were consistently demonstrable in the left eye. On the 12th day (17th

after the primary nerve section) paradoxical effects were present in the left eye before spinal cord transection midway between the 2nd and 3rd lumbar roots. At different intervals ranging from 16 to 43 minutes after the cord transection adrenalin caused both pupils to dilate but the right was always larger than the left so that the paradoxical phenomena in the left eye had evidently been suspended temporarily as the result of the lumbar transection. Six minutes after spinal transection between roots Cv and vi adrenalin caused greater and more prompt dilatation in the right than in the left pupil although in the constriction period the left was for a time the larger of the two. Eleven minutes after the cervical transection adrenalin administered in 5 per cent dextrose solution evoked paradoxical effects in the left eye. Adrenalin administered without sugar solution 10 and 14 minutes later did not evoke paradoxical effects in the left eye. Half an hour later, however, or about 1 hour after the cervical transection, adrenalin in sugar solution evoked typical paradoxical phenomena in the left eye. The right and left cervical sympathetic nerves were then cut and 5 minutes later 5 cc. of sugar solution, given intravenously, had no effect on the pupils whereas adrenalin, given in sugar solution, evoked well-marked paradoxical phenomena in the left eye. (Photograph 2.) After these experiments the animal lived about 13 hours. Six hours after death traces of paradoxical phenomena were visible in the left eye. In assessing the value of these observations the reader is reminded that they were made *17 days* after the original nerve section; that is to say, at a period when among other things regenerative and compensatory changes had been established to a considerable extent in the spinal cord and dorsal root ganglia.

In cat 3 the cord was transversely hemisected on the right side at the level of root C ii. The nerve root was considerably damaged, as shown at autopsy. Immediately after the hemisection the right pupil was larger than the left. Three days after the operation the left pupil was much larger than the right. As time went on the difference in the size of the pupils diminished somewhat but the right remained persistently smaller by 2 mm. or more than the left. Adrenalin on the 34th, 54th and 76th days caused dilatation of both pupils but the right was always considerably smaller than the left. This was considered a good case to test the effects of section of the contralateral sciatic nerve upon the constricted pupil. Accordingly on the 77th day the *left* sciatic nerve was divided. On the 12th day after the nerve section although the right pupil under all ordinary circumstances was smaller than its fellow adrenalin evoked paradoxical dilatation in the right pupil which for a

short time was the larger of the two. By the 18th day adrenalin no longer evoked paradoxical phenomena in the right eye. The scar region of the divided nerve was now excised and a portion of fresh normal sciatic nerve from another cat sutured in place between the divided nerve ends and 10 days later, although the right pupil was considerably smaller than the left, adrenalin evoked well-marked paradoxical phenomena in the right pupil which became larger for a time than its fellow (photograph 3). On the 17th day after this last operation well-marked paradoxical effects could not be obtained by adrenalin nor by mechanical asphyxiation.

Protocol. Cat 3. Male adult

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		May 11, 1919. Hemitransected spinal cord at level of root Cii on right side				
3rd			2.5	8.0	2.0	2.0
76th		Paradoxical effects absent in right eye				
		Cut left sciatic nerve				
88th		12th after section of left sciatic				
		Paradoxical effects in right eye				
94th		18th after section of left sciatic nerve				
		Paradoxical effects absent in right eye				
		Excised scar region of left sciatic and inserted transplant of fresh normal sciatic nerve				
104th		10th after insertion of transplant				
	a.m.					
	10:40	Before adrenalin	4.0	5.0	5.0	5.0
	10:40½	Adrenalin ♏ii	12.0	10.5		
	10:41	In constriction	10.0	8.0	1.5	1.0
		Paradoxical effects in right eye: photograph 3				
111th		17th after insertion of transplant				
		Paradoxical effects absent in right eye				
		Clamped trachea: pupils dilated but right always smaller than left	13.0	14.0	0.0	0.0
		Died				
		Autopsy. Scar of hemitransection involved right root Cii which was found damaged				

Alcoholic injection of one sciatic nerve. Owing to the popularity of alcoholic injections, made under the nerve sheath and sometimes into the nerve trunks for the relief of obstinate painful conditions resulting

from nerve injuries (causalgia, etc.), it was decided to test the effects of alcoholic injections into one sciatic nerve upon the pupils. The results were as consistent and striking as those obtained after sciatic section.

In cat 4 an injection of 60 per cent alcohol was made into the right sciatic nerve. Twelve days later paradoxical phenomena were in evidence in the left eye before and after cord transection between roots L ii and iii, and in death (photograph 4).

Protocol. Cat 4. Female about 3 months old

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		August 16, 1919. Injected ♏x of 60 per cent alcohol into the right sciatic nerve				
	a.m.					
12th	8:45	Before adrenalin	2.5	1.0	3.0	2.0
	8:45½	Adrenalin ♏ii	7.0	8.5	2.5	0.0
		Paradoxical effects in left eye				
	9:30	Transected spinal cord between roots Lii and iii				
	9:40		5.0	5.0	1.0	0.5
	9:40½	Adrenalin ♏ii	8.0	10.0	1.0	1.0
		Paradoxical effects in left eye				
	9:46	Gasping as dies	9.0	11.0	1.0	1.0
		Paradoxical effects in left eye; actual measurements of pupil: photograph 4				

Similar results were obtained from another animal whose right sciatic nerve had been injected with 60 per cent alcohol.

Unilateral section of the lumbo-sacral posterior nerve roots. In cat 5 an attempt was made to sever the right posterior nerve roots L vi to S ii inclusive, without injuring the anterior roots and with the least possible disturbance of the cord and the left spinal nerve roots. The attempt was manifestly successful as the animal's posture and locomotion a few days after the operation could be regarded as very little impaired were it not for the fact that the toes of the right hind limb turned somewhat under the foot in plantar flexion so that the weight was borne on their dorsal aspect; on the 2nd and 12th days after the operation adrenalin failed to evoke paradoxical phenomena in the left eye. On the 30th day adrenalin failed to evoke paradoxical effects in the left

eye although the left pupil on this day, after each injection of adrenalin, began to dilate before its fellow. On the 31st day adrenalin applied locally to each conjunctival sac failed to evoke dilatation in the pupil of either eye. On the 37th day well-marked paradoxical effects appeared in the left eye incidental to ether anesthesia and after adrenalin intravenously, and applied locally to the conjunctival sac. Transection of the cord between roots L ii and iii caused momentary dilatation in both pupils but beyond this was without apparent effect upon the paradoxical phenomena which were as readily elicited after as before the operation (photographs 5, 6 and 7).

Protocol. Cat 5. Adult, male

DAY	TIME	REMARKS	PUPILS		MEMBRANES	
			Right	Left	Right	Left
			mm.	*mm.*	*mm.*	*mm.*
		September 5, 1919. Cut right spinal roots as follows: Lvi about $\frac{2}{3}$ and Lvii to Sii about $\frac{7}{8}$ of whole distance across each root; no exposure or injury of roots on the left side				
	a.m.					
37th	9:50	Out of ether	1.5	1.5	3.0	3.0
	10:06	Under ether; wink reflex present but impaired	3.0	6.0	3.0	4.0
		Paradoxical effects in left eye: photograph 5				
	10:30½	Before adrenalin	2.5	6.0	3.0	3.0
	10:31	Adrenalin ♏ii in 1 cc. of 5 per cent dextrose solution	6.0	11.0	0.0	0.0
	10:33	In constriction	2.0	10.0	0.0	1.0
		Paradoxical effects in left eye: photograph 6				
37th	10:42	Transected spinal cord between roots Lii and iii				
	10:50	Before adrenalin	0.5	1.0	2.0	2.0
	10:53	Adrenalin ♏ii in 5 cc. of 5 per cent dextrose solution	8.0	9.0	0.0	0.0
	10:54	In constriction	4.0	9.0	1.0	1.0
		Paradoxical effects in left eye				
	11:04		1.0	2.0	2.5	2.0
	11:04½	Adrenalin ♏ii in each eye				
	11:53		1.0	9.0	1.0	1.5
		Marked paradoxical effects in left pupil; photograph 7				
	p.m.					
	4:20	Died				

In two other animals posterior nerve root section was done. The results were comparable to those just described though less striking quantitatively possibly because of slight injuries to the spinal cord and root ganglia.

DISCUSSION AND SUMMARY

After evulsion of one superior cervical ganglion intravenous injections or other paradoxical excitants do not elicit paradoxical effects before the expiration of 24 hours. Similarly after section of the sympathetic nerve in the neck paradoxical phenomena cannot as a rule be elicited before the expiration of from 5 to 7 days although under exceptional conditions they may be elicited as early as the 3rd day. The period of latency or "incubation" after section of one sciatic nerve varies from 8 to 12 days but the paradoxical phenomena may occasionally be elicited earlier, as in cat 2. The apparent prolongation of the "incubation" period in cat 5 after section of the posterior nerve roots was undoubtedly due to slight unavoidable injury of the cord or root ganglia at the time of operation. This induced on its own account pupillary phenomena which, for the time being, overshadowed the effects proper of the nerve root section. The well-known difficulty with which regeneration takes place in the posterior nerve roots after section explains the extraordinary persistence of the paradoxical phenomena as contrasted with their early appearance and disappearance after section or injury of one sciatic nerve. Following evulsion of one superior cervical ganglion paradoxical effects may be elicited in the corresponding eye for practically an indefinite period even though partial regeneration in the post-ganglionic paths may be demonstrated. After section of one cervical sympathetic nerve it is, however, easy to demonstrate that as regeneration takes place the paradoxical phenomena disappear, that is to say, in the course of a few weeks. The persistence of paradoxical phenomena so long after evulsion of one cervical ganglion is not a matter for wonder. Indeed when one considers the nature of the operation it becomes at once apparent that regeneration proceeds with difficulty and could hardly be expected ever to become complete. The condition here is analogous to what occurs after posterior nerve root section. After section or injury of one sciatic nerve the neurone bodies of the injured axones exhibit phenomena (axonal reaction phenomena, retrograde degeneration) indicative of suspension of function. These reach their maximum about

the 14th or 15th day, sooner or later, depending on circumstances, when the neurone bodies begin to exhibit changes indicating restoration of function. These changes take place in the proximal portions of the injured neurones, whether the divided nerve ends have been sutured or not, and there is evidence that at this stage and later on the regenerating neurones hyperfunction. It is noteworthy that after section of one sciatic nerve the disappearance of paradoxical effects coincides closely with the initiation of the disappearance of the axonal reaction phenomena. These facts taken in conjunction with the delayed disappearance of paradoxical phenomena after posterior nerve root section, and with the further fact that, in cat 1 after the disappearance of paradoxical phenomena these could be re-induced at will by further section or injury of the proximal segment of the divided sciatic, warrant the conclusion that after section or injury of one sciatic nerve the appearance and disappearance of paradoxical phenomena are conditioned respectively by the suspension and restoration of specific neural functioning in the related neurone bodies situated in the dorsal root ganglia.

Anderson (7), corroborating the prior finding of Lewandowsky (5), came to the conclusion that the mechanism of the paradoxical ocular phenomena after evulsion of one superior cervical ganglion, or section of one cervical sympathetic nerve, consists of increased irritability of the dilator pupillae muscle. Such increase of irritability of the effector mechanism of dilatation is the result of interruption of efferent impulses *via* the cervical sympathetic. After sciatic section or injury there occurs a similar failure of outflow of impulses to the dilator mechanism over the cervical sympathetic nerve though less in degree than in the case of ganglionectomy or section of the cervical sympathetic. The conclusion seems warranted that in the waking state afferent impulses coming from the periphery, or at least from that portion of it supplied by the sciatic nerves, are immediate factors in conditioning the outflow of impulses from the upper thoracic cord segments which govern pupil dilatation through the cervical sympathetic nerve. There is, moreover, some evidence pointing to the probability that each sciatic nerve sends impulses to the upper thoracic segments of both sides (lower cilio-spinal center of Budge) although the relation in this respect is mainly a crossed one.

The temporary disappearance of paradoxical effects in cat 2 immediately after spinal cord transection has its counterpart in the ease with which exhaustion occurs in the paradoxical mechanism at certain times. Thus after ganglionectomy or sympathetic section, toward the end of

the incubation period, or, in the case of sympathetic section, in the regeneration period just before the paradoxical phenomena disappear, repeated injections of adrenalin may fail to evoke paradoxical effects unless a period of rest be allowed to intervene. Here the effect of dextrose solution in restoring the exhausted effector mechanism is significant. Persistence of the possibility of evoking paradoxical effects after cervical and lumbar cord transection as well as after section of both cervical sympathetics, is further evidence that the paradoxical pupillary phenomena observed after section or injury of one sciatic nerve are identical in kind with the paradoxical phenomena observed after ganglionectomy or section of the cervical sympathetic nerve. This conclusion is further supported by observations made upon cat 5 in which, after section of the lumbo-sacral posterior nerve roots, adrenalin applied locally to the conjunctival sac induced marked paradoxical phenomena in the contralateral eye.

CONCLUSIONS

1. After section or injury of one sciatic nerve the appearance and disappearance of paradoxical phenomena in the contralateral pupil are conditioned respectively by the suspension and restoration of specific neural functioning in the injured neurones of the proximal segment and more particularly in the neurone bodies situated in the dorsal root ganglia.

2. In the waking state afferent impulses from the periphery, passing inward over each sciatic nerve, impinge upon the motor neurones situated in the lateral horns of the upper thoracic segments (lower ciliospinal center of Budge) conditioning reflexly the outflow of impulses that reach the pupil-dilator mechanism *via* the cervical sympathetic nerves.

3. The impingement of afferent sciatic impulses upon the upper thoracic cord segments is probably bilateral although the maximal effect is exerted upon the segments of contralateral side.

4. The paradoxical pupillary effects observed after section or alcoholic injection of one sciatic nerve are identical in kind with the paradoxical phenomena observed after removal of one superior cervical ganglion or after section of one cervical sympathetic nerve.

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FATIGUE IN FROG MUSCLE WHEN IMMERSED IN VARIOUS CONCENTRATIONS OF LIPOID-SOLVENTS; ESPECIALLY THE HIGHER ALCOHOLS

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In a recent paper (1) experiments were cited which indicate that developing sea-urchin eggs when subjected to suitable concentrations of various lipoid-soluble substances, i.e., the higher alcohols, show unmistakable rhythms of susceptibility and resistance according to the phase of physiological activity at the specific time of treatment. Such observations constitute additional evidence that a very intimate relation or correlation exists between the general physiological condition of the egg and the physical state of its plasma-membrane. The present paper is a preliminary report of experiments conducted in the light of recent advances to analyze the effects of various concentrations of the alcohols upon the resulting fatigue curves of excised frog muscles so immersed. The bearing such a study has on the theoretical and practical aspects of responses is apparent when one recalls that in any protoplasmic system, an increased (sensitization) or decreased irritability or spontaneous activity (anesthesia) may be brought about by the conditions of concentration, temperature, and the physiological state of the system.

In the case of substances, in proper concentrations, producing increased irritability, numerous examples might be cited both in plants and animals. It is well known that general nervous excitability is increased by weak doses of ether, alcohol and other active substances. Rhythmical activity such as that which takes place in cilia, or the heart beat, etc., is increased in weak solutions of alcohol and other narcotics. Carlson (2) has demonstrated that the nerve-cells controlling the heart beat of Limulus are induced to faster rhythmical action in weak solutions of alcohol, chloral hydrate, chloretone and chloroform. In experiments by Tashiro and Adams (3) similar responses in excitability in the nerve

and its output of carbon dioxide were noted when treated with low concentrations of urethane and chloral hydrate. In muscle-nerve physiology, the phenomenon of "treppe" exhibited by contracting muscle is probably due, as has been shown by Lee (4), to the stimulating action of small quantities of fatigue substances, which in higher concentrations decrease irritability. On the plant side numerous practical uses have been made of the fact that many depressant substances when administered in low concentration increase rate of growth; thus ether has been used in "forcing" plant growth by those interested in commercial horticulture.

Other striking effects of narcosis have been recorded in plants when treated with weak solutions of chloroform and ether on the side of oxygen consumption. (Tashiro and Adams cite observations of Kosinski showing that respiration in yeast cells increased in presence of 0.5 per cent ether; 5 per cent reduced respiration one-half, while 7 per cent almost stops it (4).) Rotation in plant cells has been observed within the protoplasm during the early stages of ether and chloroform narcosis, and it is recorded that the irritability of certain sensitive plants is heightened in traces of ether.

Loeb, Lillie, Torrey, Moore and others have observed striking behavior activities induced in various organisms when treated with the proper concentrations of certain anesthetics. Thus Loeb was able to produce a positive heliotropic response in Daphniae when subjected to weak solutions of alcohol and ether in strengths that vary from a third to a half of those required for anesthesia. Lillie in experiments with the marine annelid larvae (Arenicola) found that he could change the behavior from a normally positive to a negative heliotropism in various weak anesthetic substances.

On the other hand and opposite to sensitization is the phenomenon of reversible decrease in irritability or responsiveness which is anesthesia, and the vital processes that are subject to such an arrest are numerable and varied, and may be brought about in a number of different ways, i.e., mechanical, thermal, electrical or chemical. In discussing the theory of anesthesia, Lillie (7) lists a few of the vital processes so effected as follows: They include amoeboid movements; protoplasmic rotation in plant cells; all processes depending on response to stimulation, like muscular contraction and stimulation and conduction in nerve; automatic rhythmical activities like the heart beat or the motion of cilia or spermatozoa; cell-division; the artificial initiation of development in unfertilized eggs; various fermentative and oxidative processes; light-

production, e.g., by luminous bacteria; typical metabolic processes like the assimilation of carbon dioxide by plants; growth processes in plants and animals, and developmental processes dependent on growth and cell division. It is of especial significance to note that processes depending upon growth and development are included in the list, and when anesthesia is administered during proper progressive developmental stages, far-reaching consequences may result. Thus abnormalities of growth and development as well as changes in irritability may be produced under the influence of anesthesia, as Stockard (5) and McClendon (6) have shown in the production of cyclopia in developing fish eggs, and other developmental defects produced by alcohol in the case of mammals as later shown by Stockard.

It is beyond the scope and purpose of this brief introductory review of facts concerned with responses of the vital processes to attempt to discuss the cause or causes of observed phenomena, but it seems logical to infer that like manifestations of ordinary stimulation, they are in some way intimately dependent on surface-changes of the plasma-membrane. The question as to just how these surface-changes are effected is a critical one, and one that needs more careful research. Lillie (7) in his discussion of the theory of anesthesia has thrown some light on the problem and suggests that it is highly probable that the whole processes of sensitization and anesthesia are akin to the processes of stimulation. They depend upon local initiation of the excitation state which is in turn qualified by semipermeability, electrical polarization and specific metabolic activity. Hence altering the physical and chemical conditions of the membrane alters the whole physiological process. With this brief discussion, we may proceed to the consideration of the mode of experimentation described.

Methods and apparatus. The experiments about to be described were performed intermittently between April and December of the past year, the frogs being obtained in five dozen lots from a supply house in Chicago, the batches consisting for the most part of the common or leopard frog, Rana pipiens *Schreber*, sometimes called Rana virescens *Kalm*. After making all due mechanical arrangements, the specimen was killed by pithing, the brain and spinal cord destroyed, and the gastrocnemius muscle removed and placed immediately in the glass cylinder of a Harvard type muscle warmer, arranged and mounted in such a way as to allow easy manipulation in pouring solutions of desired strengths, and so connected to the inductorium and the light muscle lever as to allow the transcribing of a record on the kymograph when the muscle was

stimulated by the intermittent induction shocks. Immediately above the muscle lever was mounted a Deprez double electric signal marker, the upper marker being connected through wires with a Harvard type chronometer beating off intervals of a half-minute; the lower being connected through wires with the primary circuit through a Harvard type vibrating interrupter beating seconds (a make and break during the second so that the muscle actually received through the inductorium two stimuli per second). The current used through wires was from electric storage battery, type "D," cells maintained approximately at full charge (1.225 s. g.) delivering a voltage to the inductorium of about 2 per cell. Usually two cells were used to impel the induction coil of the interrupter, with one cell furnishing the current to the inductorium and the muscle which of course was connected to the inductorium through the terminals of the secondary coil. The secondary coil in all experiments was placed over the primary so that it was 2 cm. from its fully closed position, thus delivering to the muscle practically its maximal induction. After extended preliminary experimentation it was found that about the optimum mechanical advantage in terms of leverage for the muscle lever consisted of having the muscle attachment 8 mm. from the pivotal fulcrum opposed by a 30 gram weight placed 14 mm. on the opposite side, the entire length of the writing arm being 150 mm. from the pivot. The kymograph used in recording was of the modified Harvard slow driving, long paper type, so provided with a fan that it made one revolution in about 17 minutes. All records shown in the subjoined plates were made with the drum revolving at this uniform speed so that from this factor they are comparable.

During the experimentations care has been exercised to keep the various mechanical and thermal conditions as constant as possible; the temperature has not been allowed to fluctuate more than 2 degrees at any time, usually the room temperature being kept at approximately 21°C. during all the experiments. The various concentrations of fluids used were made up in advance according to computed volumes per cent, and placed in small, cork-stoppered flasks of 150 cc. capacity, so that at the time of experimentation they were approximately that of room temperature. The manner of attachment of the muscle to the electrodes was carefully observed, and consisted of inserting the needle in the tendon achilles just at the junction of the fascia of the numerous muscle bundles for the lower contact; the upper end of the muscle being made secure by piercing the tendonous fascia at the knee joint between the distal end of the femur and the proximal end of the tibia. This

precaution to make secure the electrodes was found to be imperative since otherwise the inertia of the falling weight on the writing lever opposing the muscle would invariably alter the elasticity of the muscle and complicate the curves, especially in the initial excursions of the lever.

By closing the short ciruit bar on the key, the interrupter in the primary circuit activated intermittently the signal magnet and at the same time sent the make and break shocks to the muscle through the inductorium. It is assumed that by keeping constant the relative positions of the primary and the secondary coils, the resulting successive induced shocks which reach the muscle will be approximately of the same strength throughout any series of experiments. This factor, of course, is a very important one since the responses of skeletal muscles when supplied with gradations of current are markedly affected in a number of its phases, especially fatigue, as has been recently shown by Pratt (8). Assuming the factors just mentioned to be fairly constant throughout the series of experiments to be described, there is yet another variable not easily controlled, that of metabolic variability in the individual muscles. Selection as to equality in size and weight of the experimental muscles has been the only available criterion on this point. From comparative uniformity of the resulting curves obtained on repeated trials it would seem that this criterion could be relied upon within the limits of experimental procedure.

The alcohols used throughout the experiments were obtained from different sources; the methyl, ethyl and n-butyl were redistilled in the organic laboratory here at the college; the n-propyl was obtained from Merck; n-amyl, nonyl, decyl were obtained from the Eastman Kodak Laboratories, the last two named being put on the market as "technical" and were not used in this series of experiments. The secondary octyl was purified by the Eastman Kodak Company while its isomere capryl was "practical" and was redistilled here in the organic laboratory. Eastman's "technical" heptyl alcohol was used but for the most part records with this alcohol were unsatisfactory, probably because of its oily nature.

Experimentation. Ranges in suitable concentrations of the alcohols vary widely according to the specifically desired physiological result. For example, Overton in his study of narcosis of tadpoles found the range to lie between 0.57 mol per L. for methyl to 0.0004 mol per L. for octyl. Contrasted to these concentrations are observations by Führer and Neubauer in producing hemolysis where the range varied from 7.34

mols per L. for methyl, to 0.004 for octyl, and Vernon's range in the destruction of indophenol oxidase was even higher in the first four members of the series that he tried, being 10.5 to 14 mols per L. for methyl to 0.032 to 0.9 mol for butyl. These last figures correspond very closely to those found to apply to isocapillary solutions, e.g., from 14.0 mols per L. for methyl to 0.14 mol for amyl. On this basis it was necessary to compute ranges of concentrations over rather wide limits, and to select therefrom those concentrations that promised to produce the desired physiological effects, i.e., bearing in mind that solutions of strengths above the optimum concentration would likely be too toxic and would result in depression or inhibition (anesthesia) of the muscular response, and on the other hand those below would probably have a stimulating (sensitization) effect.

TABLE 1

ALCOHOLS	CONCENTRATIONS OF ALCOHOLS				
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
Methyl	Strong	50	29.1	20.8	12.4
Ethyl	95.0	50	17.2	9.1	7.4
N-Propyl	Strong	Saturated	13.3	5.9	3.7
N-Butyl	Strong	Saturated	4.7	3.4	1.7
N-Amyl	Strong	Saturated	1.1	0.5	0.2
Heptyl	Strong	Saturated	1.6	0.7	0.4
S-Octyl	Strong	Saturated	0.62	0.29	0.15
Capryl	Strong	Saturated	0.50	0.15	0.05

The alcohols were diluted with distilled water. Under the conditions of experiments the muscles when immersed in physiological salt solution gave the typical initial contracture (treppe) phase for four or five strokes of the lever, followed by a decided relaxation phase longer in duration during which time an increased responsiveness was apparent. The second contracture phase develops regularly after the space of about a half a minute, and the muscle shortens in such a way to show a fall in the general contour of the top of the curve, accompanied by a gradual rise in the lower portion of the record. A typical record of this kind is shown in figure 12. A similar series of phases occurs when the muscle is stimulated in air, there being in this case no impediment in resistance to overcome by the muscle in its contraction, the resulting curve is comparatively larger, as shown in figure 6. The superimposed lighter curve in this case results from the "make shocks" while the larger and darker area is caused by the more intense "break shocks."

The mean time necessary to fatigue a muscle either in physiological salt solution or in air was found to be about 4 minutes, as recorded by the chronometer beating half-minutes, basing the calculation arbitrarily upon the point at which the lower margin of the second contracture phase begins its final descent.

Methyl alcohol. Strong methyl alcohol is markedly stimulating during the first few induced contractions (perhaps a dozen strokes of the lever), but its toxic effect is immediately noticeable and the progress of the second contracture is rapidly completed, the whole taking place in less than 2 minutes. A typical curve using this alcohol is shown in figure 1.

Methyl alcohol in strength of 50 vol. per cent (fig. 2) produces a rather uniform curve. The initial contracture phase is noticeably lacking, with an accompanying relaxation phase apparent as indicated by a drop in the excursion of the writing point. The second contracture phase is similar in form and duration to that produced by alcohol of great strength, fatigue resulting in this case in about $1\frac{3}{4}$ minutes.

Alcohol of 29.1 vol. per cent, as typically shown in figure 3, is decidedly stimulating during the initial contractions, but produces rapid fatigue once the secondary contracture phase is entered upon, the fatigue process lasting only $1\frac{1}{2}$ minutes.

Of all the concentrations tried with this alcohol it would seem from the records obtained that 20.8 vol. per cent gives the best combination of initial stimulation followed by rapid fatigue indicating toxicity and alcohol of approximately this concentration may perhaps be considered as about on the border, possessing favorable penetrating qualities. The muscle reached its final contracture phase in about $2\frac{1}{4}$ minutes and produces an interesting curve as shown in figure 4.

Methyl alcohol of 12.4 vol. per cent is obviously below optimum concentration to produce rapid fatigue. It, like the higher grades of this alcohol, stimulates noticeably during the initial contractions, proceeds to assume contracture proportions. A typical curve produced by muscles thus subjected is shown in figure 5, and from comparison is similar to one produced in normal salt solution (fig. 12).

Ethyl alcohol. Strong ethyl alcohol has a surprising stimulating effect upon muscles undergoing the fatigue process, so that the onset of the second contracture phase is very slow and remarkably gradual. The treppe effect brought about is perhaps more conspicuous here than in any other cases tried. Once the final contracture phase is produced, however, it is maintained for a long time with scarcely any decline ap-

Plate 1. *1.* Typical fatigue curve resulting from stimulation of muscle immersed in strong methyl alcohol. Note the initial relaxation phase is almost lacking, and the temporary stimulating effect as evidenced by the height of the first few contractions.

2. Fatigue curve of muscle immersed in 50 vol. per cent methyl alcohol. The early relaxation phase is here beginning to be conspicuous and is somewhat prolonged.

3. Typical curve when immersed in 29.1 vol. per cent methyl alcohol. Relaxation phase more pronounced, conspicuous treppe, but rather rapid onset of secondary contracture.

4. Curve resulting on immersion in 20.8 vol. per cent methyl alcohol. This concentration perhaps may be regarded as lying in a relative range of concentration where phases are well balanced in modification. A rather prolonged initial relaxation with long constant strokes, a uniform secondary contracture terminating in a reversible decline toward the end.

5. Curve resulting from immersion in the weakest alcohol used, 12.4 vol. per cent. Somewhat stimulating as evidenced by the early relaxation phase, the height of the excursions and the duration of upper margin of the plateau of secondary contracture.

6. Comparative curve resulting from stimulating muscle in air. Note the height of excursions, the initial relaxation phase, and the gradual and uniform development of secondary contracture. The whiter area inside is caused by the less intense "make" shocks.

7. Typical strong ethyl alcohol curve. Practically no initial relaxation phase, gradual onset of secondary contraction, a conspicuous treppe and a sustained contracture plateau.

8. Typical curve using 50 vol. per cent ethyl alcohol. Phases in marked contrast to proceeding, initial relaxation, rapidly developing secondary with maintained plateau becoming reversible.

9. Typical curve using 17.2 vol. per cent ethyl alcohol. Stimulating with modified toxic effect, note the rather prolonged initial relaxation followed by rapidly developing secondary with sharply declining plateau.

10. Typical curve using 9.1 vol. per cent ethyl alcohol. Stimulating with more slowly toxic effect. Gradual onset of secondary contracture and maintenance of plateau and reversible decline.

11. Typical curve using 7.4 vol. per cent ethyl alcohol. Not greatly different from preceding.

12. Typical curve obtained under same experimental conditions as all the others but in physiological salt solution, 0.7 vol. per cent. Note the initial relaxation phase accompanied by treppe at the top, the gradual onset of secondary contracture, and a slowly reversible decline, with maintained gradual inclined plateau.

13. Characteristic curve resulting from immersion in strong propyl alcohol. Primary contracture very evident with no reversal to relaxation whatsoever, immediate onset of secondary contracture, terminating in irreversible plateau.

14. Typical curve using saturated solution of propyl alcohol. A slight assumption of initial relaxation, no treppe, uniform plateau almost irreversible.

15, 16, 17. Typical curves using 13.5, 5.9 and 3.7 vol. per cent propyl alcohol. First two maintaining almost an irreversible plateau, the last showing a rapid decline.

Plate 2. *18.* Typical curve in strong butyl alcohol. Practical absence of initial relaxation phase, rapid onset of secondary contracture with maintained final plateau.

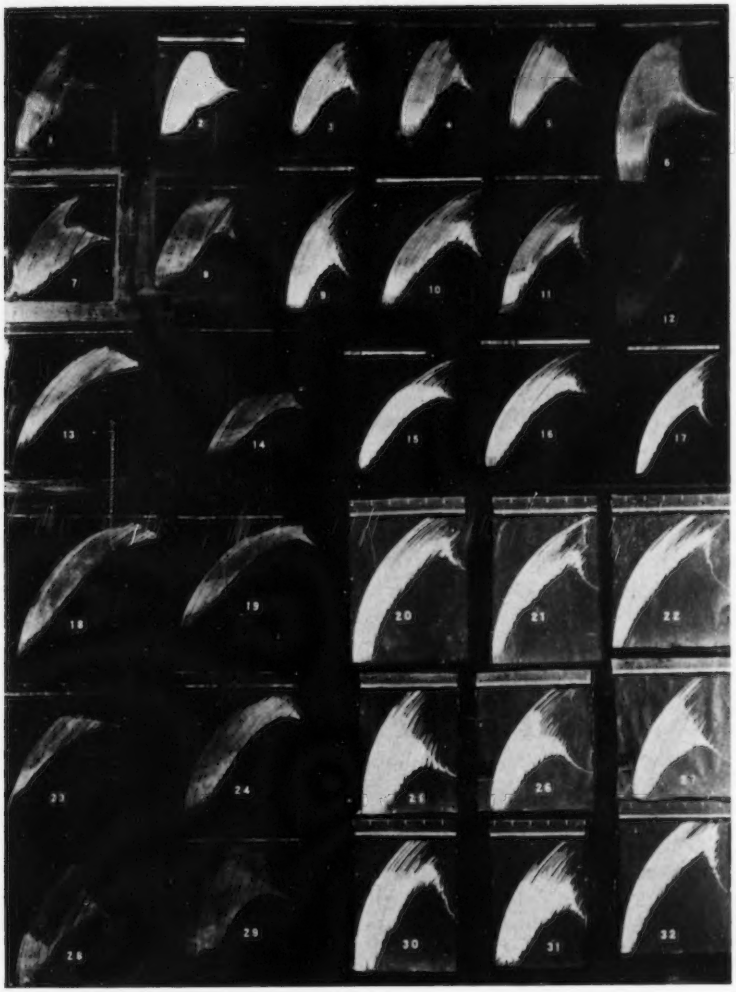
19. A suggestion of initial relaxation phase in saturated solution of butyl alcohol, with maintained horizontal plateau which is slowly reversible.

20, 21, 22. Curves resulting on immersion in 1.7, 3.4 and 4.7 vol. per cent butyl alcohol, respectively. By a mistake the order in strengths was reversed in the labeling. *22* is the strongest (4.7), *20* the weakest (1.7). Characteristic in reversible decline.

23. Typical curve in strong amyl. Note similar contours to those obtained with propyl and butyl alcohols of equal strengths.

Plate 1

Plate 2



24. Typical curve obtained in saturated solution of amyl alcohol. Comparatively similar to equivalent strength of propyl and butyl alcohols.

25, 26, 27. Curves produced in solutions of 1.1, 0.5 and 0.2 vol. per cent amyl alcohol, respectively. Characteristically different from those of similar strengths of propyl and butyl, and more closely resembling those of weak solution of methyl alcohol, *3*, *4* and *5*.

28. Curve resulting from immersion in secondary octyl alcohol and in sharp contrast to those in strong solutions of its predecessors, propyl, butyl and amyl alcohols. Notice an initial relaxation phase which occurs nowhere else in curves of strong alcohols, and almost as extensive as that produced in salt solution, *12*, in fact, in all its details it is almost a duplication of curves produced in salt solution.

29. Saturated solution of secondary octyl alcohol. It contrasts sharply with curves obtained from similar concentrations of propyl, butyl and amyl alcohols, and its nearest simile is that produced by comparative strength of ethyl alcohol, *8*.

30, 31, 32. Curves of secondary octyl alcohol of weak strengths, 0.62, 0.29 and 0.15 vol. per cent, respectively. These as can readily be seen are easily comparable to those obtained from similar strengths of butyl, and to a less degree of propyl alcohols.

parent. Muscles in this strengh fatigue quite as slowly as those submitted to physiological salt solution. Figure 7 shows a typical record obtained with strong ethyl alcohol. There is little initial relaxation apparent such as develops in concentrations of lower strengths.

Ethyl alcohol of 50 vol. per cent is remarkable in that it produces a sustained relaxation phase of uniform responsiveness as evidenced by the plateau form of the record (fig. 8), accompanied by a rather uniform base line. Once the fatigue is brought about, the second contraction phase appears rapidly as evidenced by the uniform ascent of the lower margin of the described curve. Contracture, however, is not prolonged as in strong alcohol, but begins immediately and is regular as in the case of normal fatigue.

The relative effects of subjecting muscles to the three lower concentrations of ethyl alcohol, namely 17.2, 9.1 and 7.4 vol. per cent, may best be seen by referring to typical curves shown respectively in figures 9, 10 and 11. That an increase in sensitization is caused by a decrease in concentration is strikingly apparent here. The highest of these three concentrations brings about almost immediately the onset of secondary contracture, so that the upper margin of the curve drops regularly while the lower margin has an accompanying uniform rapid rise, the whole fatigue process taking only $1\frac{1}{2}$ minutes to complete. Contrasted to this type of curve are those of lower concentrations, where the secondary contracture phase is gradually induced and maintained at a relatively higher and more uniformly regular level. In both of these the time necessary to fatigue is better than $2\frac{1}{2}$ minutes, and the increased responsiveness of the muscle is evidenced by the increased height of the recording lever. Once initiated, the decline in the secondary relaxation is rapid in all three cases, which is in marked contrast to curves produced by practically all concentrations of propyl or butyl alcohols (see figs. 13 to 19 inclusive) or in the case of strong methyl and ethyl alcohols (figs. 1 and 7). This point, it would seem, is an important one in the analysis, since it implies that once the stimulating effect has run its course, changes are brought about in the muscle which in turn now reverse the process and something akin to anesthesia ensues so that the relaxation is induced as rapidly as contracture was at first effected.

Propyl alcohol. Propyl alcohol in all ranges of concentrations except one (3.7 vol. per cent, fig. 17) gives striking and characteristic fatigue curves as may be seen by referring to figures 13 to 17. On being immersed the muscle immediately begins to shorten and this is accompanied by an increased sensitization in most of the concentrations tried.

This tendency to immediate shortening is especially noticeable in strong and saturated solutions, but is more or less conspicuous in the lower concentrations. In strong propyl alcohol there is only a suggestion of the treppe, the noticeable phase being the uniform secondary contracture which is maintained at a relatively high level (fig. 13). Saturated propyl forms a plateau with accompanying contracture (fig. 14) with maintained high contracture phase. In strengths 13.3 and 5.9 vol. per cent the resulting curves are very similar to one another as shown in figures 15 and 16, respectively. In the lowest concentration tried, 3.7 vol. per cent, the muscle fatigues uniformly and quickly, but after the completion of the second contraction phase it goes into relaxation rapidly so that the resulting curve (fig. 17) is in this respect exceptional to the other concentration of this alcohol, but comparable to the lower strengths of ethyl alcohol noted above.

Butyl alcohol. Strong and saturated solutions of this alcohol give curves (figs. 18 and 19) which are typical and which are comparable with similar strengths of butyl alcohol just described. Contracture starts almost immediately on being stimulated and is maintained at a remarkably uniform rate with almost no relaxation phase at the end of the fatigue cycle. The three solutions of weaker strength of this alcohol tried, namely, 4.7 (fig. 22), 3.4 (fig. 21) and 1.7 (fig. 20) vol. per cent gave typical curves similar to each other and to the weakest concentrations of ethyl and propyl alcohols. This would seem to indicate that the differences in range as computed were not sufficient to give decided differences in effective physiological responses sufficient to analyze. All tend to have a definite comparable initial relaxation phase as indicated by the drop in the writing point in the early progress of the curve, and all on the completion of the second contracture phase have a rather rapid onset of secondary relaxation as indicated by the abrupt descent at the terminal portion of the curves. It is very evident, too, that these concentrations have an exhaustive stimulating effect since fatigue once started proceeds rapidly, and that in this respect they are more potent than the three low grades of either methyl and ethyl alcohols and even more so than comparable toxic strengths of its predecessor, propyl alcohol.

Amyl alcohol. In strong and saturated concentrations amyl alcohol gives curves similar to those of comparable strengths of both propyl and butyl alcohols and decidedly in contrast to comparable strengths of methyl and ethyl alcohols. With strong amyl alcohol the first contracture phase is produced immediately on being stimulated, as shown

in figure 23, and proceeds gradually to produce the secondary contracture (see fig. 13 for comparison with propyl alcohol). The plateau of secondary contracture is with this alcohol more pronounced and extensive than is the case with propyl or butyl alcohols. The three lower concentrations used, 1.1, 0.5 and 0.2 vol. per cent, strange to say, give curves (figs. 25, 26 and 27, respectively) which in all details more closely resemble the three comparable strengths of methyl alcohol than any other concentrations of its predecessors (see figs. 3, 4 and 5 in comparison).

Hexyl and heptyl alcohols. No hexyl alcohol was available at the time of experimentation so that no records were obtained. Heptyl alcohol was at hand and its effects on fatigue in different concentrations somewhat explored but due to incompleteness of records no comparisons of value can be made at this time.

Octyl alcohol. Two isomeric solutions of this alcohol were tried, a secondary octyl from a purification process of the Eastman Kodak Company, and a so-called capryl alcohol from the same source, redistilled here. Little difference physiologically could be seen in using either in similar concentrations. Both give curves (figs. 28 and 29) in strong and saturated solutions which are remarkable in their similarity, not to their immediate predecessors of equal strengths, but to ethyl alcohol. The three lower concentrations, however, give curves (figs. 30, 31 and 32) which are fairly comparable to similar concentrations of butyl alcohol, with perhaps a less marked similarity to the weakest strength of ethyl and propyl alcohols. These considerations when taken together with certain other data seem to point to the fact that octyl alcohol in various concentrations does not exert as striking penetrating qualities as butyl, propyl or amyl alcohols. From incomplete records obtained in use of heptyl it would seem that this alcohol also is in a similar category.

SUMMARY

Making use of proper laboratory apparatus herein described in which experimental conditions may be kept reasonably constant, records were obtained in the development of fatigue in the gastrocnemius muscle of the frog while being subjected during its stimulation to certain computed concentrations of various alcohols.

The ranges of concentrations explored may be briefly tabulated; strong and saturated solutions of methyl, ethyl, propyl, butyl, amyl, heptyl, octyl and capryl, with computed gradations in three series of

each, varying from 29.1 methyl, to 0.62 vol. per cent octyl; 20.8 vol. per cent methyl, to 0.29 vol. per cent octyl; and 12.4 methyl to 0.15 vol. per cent octyl, respectively.

On comparative analysis of the various phases of these curves certain inferences can be drawn as to penetration and its resulting effects on the muscle of the different alcoholic concentrations used, both as to stimulation or sensitization and inhibition or anesthetic effects. Strong concentrations in general give remarkably uniform modifications in phases of contraction, especially in producing immediate contracture which merges without interruption into irreversible secondary contracture. Certain weak solutions in general are markedly stimulating as evidenced by initial and somewhat prolonged relaxation phase followed by a reversible contracture phase which is very pronounced. Certain predictable differences were obtained in concentrations between the two extremes.

The evidence presented would seem to indicate that muscles when undergoing the process of fatigue are qualitatively susceptible to differences in concentration of the medium with which they are surrounded. This implies that an intimate relation exists between the physical state of the muscular envelope (plasma-membrane) and the changing physiological conditions within.

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AMOEBOID MOVEMENT, TISSUE FORMATION AND CONSISTENCY OF PROTOPLASM

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In a series of preceding papers we have attempted an analysis of the conditions which lead to the changes in the blood cells of Limulus after they have left the body of the animal. These changes concern the clot formation, the shape of the cells, the granules, amoeboid movement and the consistency of the cell (1). In this paper we shall analyze the effect of changes in osmotic pressure and constitution of medium, the effect of changes in temperature and of the character of the cellfibrin on the amoeboid movements in their dependence upon the consistency of the cell, and we shall discuss some wider bearings which our experiments may have on the theory of tissue formation and tissue movements in general.

We used the method described in a previous paper. We prepared experimental cellfibrin tissue and observed the migration of cells from this tissue in the hollow slide. In order to eliminate the effect of variable factors, such as variations in the size of the piece, in the amount of fluid added and others, we made a very large number of preparations.

It will not be necessary to review again the literature on amoeboid movement. This has been done repeatedly especially by Rhumbler. The most generally accepted view is that amoeboid movement depends on primary changes in surface tension in a liquid medium. This view has again been upheld quite recently by Tait (2).

Effect of osmotic pressure of medium on rate and amount of outgrowth. Of all the media used the outgrowth was found best in Limulus serum, at least in the beginning of the experiment. On the second day, or perhaps somewhat earlier, the growth may become equally good or even somewhat better in a m/2 NaCl solution. In solution of a higher osmotic pressure than m NaCl no outgrowth occurs. In m NaCl only a trace of growth is seen. In $\frac{3}{4}$ m NaCl there is a distinct outgrowth,

but it is usually considerably less than in solutions of $\frac{5}{8}$ m NaCl — m/2 NaCl. In these solutions generally the optimal growth is reached. In m/4 NaCl it is in the majority of cases less good than in m/2 NaCl, but occasionally it may be equally good; only in a very small minority of cases was it better. In m/6 and m/8 NaCl it is much less good than in m/2 NaCl.

In interpreting these results we must consider the probability that gradually substances are extracted from the tissue and added to the solution. This not only would tend to increase the osmotic pressure of the solution, and thus shift the optimum toward the originally hypotonic solutions, but in addition it might tend to add to the NaCl solution substances which render the constitution of the solutions more similar to the body fluid. In determining the effect of the medium on the tissue we have to take into consideration not only the width of the zone in which migrating amoebocytes are found, but also the density of the field (the number of outgrown cells) and the character of the cells, the preservation of the granules, the character of the pseudopodia and the intensity of the spreading-out process.

The influence of osmotic pressure on the character of pseudopods and amoeboid movements. The character of the pseudopods and amoeboid movement in the amoeboyctes of Limulus is not fixed but is variable and the variations depend upon a number of variable factors, such as character of cellfibrin tissue, temperature, difference in chemical composition of the surrounding medium and osmotic pressure.

We shall here discuss the typical effects caused by changes in osmotic pressure in NaCl solutions leaving the other factors approximately constant. This will be followed by a discussion of the other variable factors.

In m NaCl solutions the pseudopods are sharp, fine, threadlike or they are sharp tongue pseudopods. In $\frac{3}{4}$ m NaCl the cells are usually relatively small, contracted, granular. The granules appear small and are close together. The cells begin to spread out in contact with the glass, but the intensity of spreading out and the number of spread-out cells is restricted. NaCl tends gradually to lead to a solution of the granules.

Usually the pseudopods are here fine and long, threadlike; sharp pointed tongue pseudopods are also quite common. The thread pseudopods are very often multiple and very long, they may show a tree-like ramification so that structures not unlike glia cells are produced; or the appearance may be similar to bipolar or multipolar ganglia cells. At

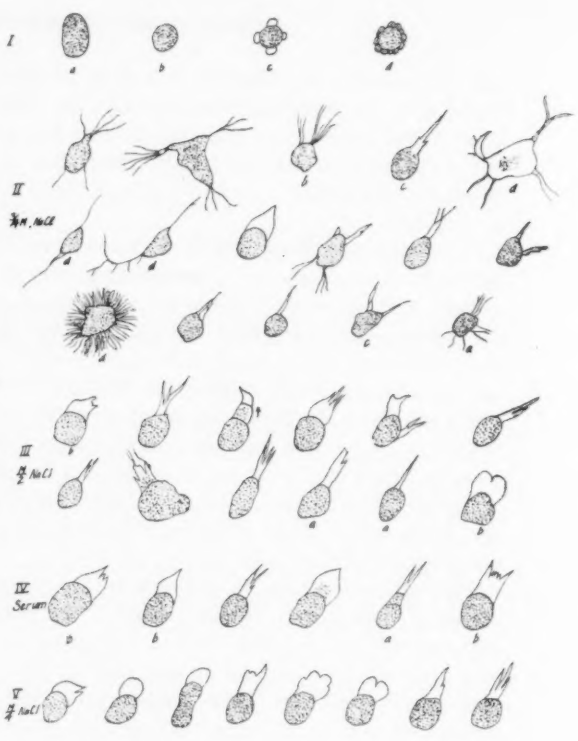
The figures are more or less diagramnatic and show mainly the character of the pseudopodia in different media.

I. *a*, Circulating blood cells in the normal animal; they are flat plates. *b*, Rounded off cell after it has left the body. *c* and *d*, Effect of a sharp rise in temperature.

II. Hypertonic medium. *a* and *b*, cells with thread pseudopodia; *c*, sharp tongue pseudopodia; and *d*, cells resembling ganglia and glia cells.

III and IV. Isotonic medium. *a*, sharp tongue pseudopodia; *b*, broad tongue pseudopodia.

V. Slightly hypotonic medium. The broad tongue pseudopodia prevail.



VI. Markedly hypotonic medium. *a*, balloon pseudopodia; *b*, court pseudopodium; *c*, transitional pseudopodium; *d*, broad tongue pseudopodia; *e*, successive balloon pseudopodia with immigration of granuloplasm; *f*, multiple balloons.

VII and VIII. Action of potassium in isotonic and slightly hypotonic solution. *a*, Structures resembling ova with fertilization membranes; *b*, pseudopodial processes developing in such cells; *c*, cell with half-moon; *d*, cell with circus movement; *e*, transitional form.

the angles of triangular cells processes are attached which are very long, become finer in their distal parts and split dichotomously. In other cases we find very fine long dichotomous threads sitting on broader tongue pseudopods. In certain cases we may even find broad tongue pseudopodia, drops and small balloons in $\frac{3}{4}$ m NaCl solutions. This occurs especially if cellfibrin tissue is used which has a very low degree of resistance and if the tissue has been exposed to the temperature of the incubator. But even in these cases the drops and balloons are usually smaller than in non-hypertonic solutions and we are apt to find transitions to tongue pseudopodia. After having been kept for several days, even in the ice chest, many cells are flat, spread out, hyaline, and these cells usually show long branching pseudopods. These long threadlike pseudopods change even $\frac{3}{4}$ m NaCl solution, they may be partly drawn in and be sent out again at other places. On the whole the movement of the granuloplasm and of the hyaloplasm tends to be slow in this solution, and the granuloplasm is apt to move as a connected mass, although occasionally some granules may advance isolated into the pseudopod. We find here also successive formation of tongue pseudopodia. The granules move not only into the broader pseudopods, but occasionally also into fine threadlike pseudopods, the interior of which is less consistent than the periphery.

If we observe the cells emigrating from a piece of cellfibrin tissue in $\frac{3}{4}$ m NaCl solution, in which the temperature has been raised by placing the specimen soon after it has been made, under the microscope near an electric lamp, we may notice the appearance of very sharp pseudopods with which the cells emigrate from the tissue. In beginning however the pseudopods may not yet be quite so sharp as later, when the solution has had time to act on the cells and when perhaps the hypertonicity of the solution has been still further increased through substances extracted from the tissue.

In cells which have become more or less hyaline in the solution we may notice a framework of hyaline glistening, apparently relatively solid hyaloplasm from which protrude long fine pseudopods of a similar consistency, and which includes a vacuolar space in which the nucleus is situated. Some granules which are left in the cell move in contact with the hyaline framework or with fibers which are suspended in the vacuole, and it appears almost as if the granules were attached to the more solid structures. However, they become free and move in the vacuole, as soon as the fibers are dissolved. In $\frac{3}{4}$ m NaCl the amoebocytes show only a slight agglutination; the marked clump formation as we find it

especially in serum but also in some other solutions, is here usually absent.

In $\frac{5}{8}$ —m/2 NaCl solutions the cells are at first contracted granular. Gradually they spread out in contact with the glass and become hyaline; the cells which are not in contact with the glass remain contracted for a longer period and they keep their granules for a considerable time, but ultimately some of these cells may also become hyaline. The extending cells are at first still granular. Agglutination of cells into clumps is here usually more marked than in $\frac{3}{4}$ m NaCl, but less so than in blood serum. In m/2 NaCl the pseudopods are usually sharp, medium or broad tongue pseudopods with a prevalence of the sharp type. Some thread pseudopods may also occur. Under the same conditions, when drops and balloons appear in $\frac{3}{4}$ m NaCl solution, they may likewise appear in m/2 NaCl. This is especially the case whenever less resistant tissue is used and if the tissue had been kept at a higher temperature. But in m/2 NaCl these drop and balloon pseudopods are more frequent than in $\frac{3}{4}$ m NaCl and they may even occur spontaneously at room temperature in older specimens and especially in the most advanced cells which are more directly exposed to the salt solution. If we observe under the microscope the cells emigrating from the tissue in a freshly prepared specimen near a heat emitting lamp circus movement may occur, but it is very rare; it occurs under conditions which favor balloon formation.

Three-eighths m NaCl and m/4 NaCl: Only a few experiments were carried out with $\frac{3}{8}$ m NaCl, in the majority of cases m/4 NaCl was used. Here the same variety of pseudopodia and movements are found as in m/2 NaCl, namely, sharp, medium and broad tongue pseudopodia, drops, balloons and half-moons; but the relative frequency with which these different types are observed is different in m/4 and m/2 NaCl. Broad tongue pseudopodia, drops, balloons and half-moons are here relatively more frequent. The same factors which favor the formation of the latter in isotonic solutions have the same effect in m/4 NaCl; but they are more effective in the latter solution. These factors are length of time, during which the cells are exposed to the solution, higher temperature and the use of less resistant tissue. After exposure to the incubator temperature drops and balloons are apt to appear; they are more frequent in the peripheral, most advanced cells, than in the central cells. Circus movements may appear, but are rare.

M/6 and m/8 NaCl solutions: We can treat these two solutions jointly; their effect is essentially the same, although it is more pronounced in the latter solution.

Tongue pseudopodia as well as drops, balloon pseudopodia, half-moons and courts may be seen, but the balloons, drops, courts and half-moons are here relatively much more frequent than in the less hypotonic solutions. Balloons, courts and half-moons may be considered as the typical pseudopodia in these hypotonic solutions, but broad and even sharp tongue pseudopodia may also occur; they are the more frequent, the more resistant the tissue is, the less the time is which has elapsed since the specimen was exposed to the solution, the less the tissue was exposed to a higher temperature. We observe here occasionally *pari passu* movement of the granuloplasm into the half-moon; thus a partial extension of the cell may take place. In this condition the granuloplasm enters the pseudopods at once. The cell on the whole in this solution is rather large, the granules are well separated from each other and they are large. In consequence the cells are apt to appear as dark granular cells under the low power of the microscope. The cells are soft and the granuloplasm usually moves rapidly into the balloons. Successive balloons also occur, and the cell as a whole may show locomotion by these means.

If we observe a piece of tissue in these hypotonic solutions near a lamp at a higher temperature, we notice in the first hour and a half after the specimen has been made, an emigration of granular cells with large balloons, which are usually directed toward the outside and into which the granuloplasm moves. The large majority of the cells show these balloons in the first 90 minutes. Then the number of cells which emigrate with tongue pseudopodia becomes more frequent; and a number of cells may begin to show broad tongue pseudopodia or transitions between tongue pseudopodia and balloons in the solution. Under certain conditions sharp and broad tongue pseudopodia may even predominate in these solutions. It is possible that gradually substances are extracted from the tissue which increase the osmotic pressure of the solution and make it also otherwise more adequate for the blood cells.

A broad tongue pseudopod may explode into a balloon and a balloon may become transformed into an irregular tongue pseudopod. Thus transitional forms are produced. Slight circus movements are also occasionally observed. The same cell may alternately show broad tongue pseudopodia and balloons, and balloons may also be polarized, appear preferably at a certain pole of the cell. Balloons appear here much earlier than in solutions with a higher osmotic pressure and again they are more frequent in less resistant fibrin and at higher temperature.

If we lower the osmotic pressure of the medium still further by placing the tissue in distilled water, no outgrowth occurs. As I have shown previously blood cells do not under those conditions show those phenomena which we observe in hypotonic salt solutions, but the water changes the cell substance throughout in such a way that amoeboid movements are no longer possible. The cell becomes round, polygonal or leaflike and the majority of granules disappear; only exceptionally may we find a cell which shows a very broad or balloon pseudopod under those conditions. This is probably at places where through extraction of substances from the tissue the osmotic pressure of the fluid has been raised.

In blood serum of Limulus the typical pseudopodia are the various kinds of tongue pseudopodia; but here also some drops or even halfmoons may form under certain conditions. The factors which favor the appearance of round pseudopods are the same as those mentioned previously: namely, spontaneous changes taking place in the cells, after a certain time—usually several days—has elapsed, the use of less resistant tissues and the use of higher temperature.

If we compare the behavior of the cells in Limulus serum and approximately isotonic NaCl solutions (m/2 and $\frac{5}{8}$ m), we find the following differences. In serum the tongue pseudopods are on the average broader than in NaCl solutions. In serum the cells tend to spread out with granules intact, while in NaCl solutions the cells are either more contracted, or if they do spread out especially in contact with the glass they lose part of their granules earlier than in serum. In serum the cells are agglutinated in small clumps to a greater extent than in NaCl solutions, where they are distributed more evenly.

Effect of various salt solutions on the amoebocytes. In our former investigations we studied the effect of various substances on the amoebocytes of Limulus. At that time we considered especially the cell consistency and the fate of the cell granules and only secondarily were we concerned with the character of pseudopods in these solutions. In our recent experiments we studied more particularly the effect of these solutions on the amoeboid movements. So far we gave special attention to the action of KCl, because we found previously that the various potassium salts seemed to have a softening influence on the protoplasm of the blood cells. We also noticed that the pseudopodia were less sharp in isotonic KCl than in NaCl solutions.

Effect of m/2 KCl on the amoeboid activity. If we place a piece of tissue in a m/2 KCl solution on a cover glass, we find only a very lim-

ited outgrowth of cells. The majority of the cells are round and granular; some of the cells become hyaline. Pseudopodial activity is limited; we may find sharp and broad tongue pseudopods but also somewhat swollen cells with courts, half-moons and drop pseudopodia, and others presenting structures resembling eggs with fertilization membranes; furthermore pseudopods transitional between tongue and balloon or drop pseudopods. Here also the character of the tissue is of some effect, and also the temperature effects are noticeable, but even in tissue kept in the ice chest some results of softening, as the formation of courts can be observed. If the softening effect in the cytoplasm is sufficiently marked, Brownian movement of the granules may be seen in the cells.

If we use $\frac{3}{4}$ m KCl solution, which is somewhat hypertonic, we observe round granular or hyaline fixed cells in contrast to a $\frac{3}{4}$ m NaCl solution, where the cells show very sharp fine pseudopods. The most characteristic effects of KCl however are obtained in m/4 solution which is somewhat hypotonic. Here the cells are usually round, rather large; the granules are larger and distinct and the cell appears dark, granular. The outgrowth in this solution is usually slight, similar to that in m/8 NaCl. If we observe under the microscope the outgrowth of cells from the tissue near the lamp soon after the specimen has been made, we find many cells with large balloons which are directed outward, away from the tissue. The granuloplasm moves into these balloons.

Other cells move out with irregular balloons which form transitions to broad tongue pseudopods. Here also the granules move into the balloons rather rapidly. If we observe the cells somewhat later, after they have been in this solution for some time we find in addition to the balloons cells with half-moons, courts; other cells resemble eggs with fertilization membranes. There are occasionally some cells with tongue pseudopodia, or transitional forms between balloon and tongue pseudopodio, but usually they are absent. A court pseudopodium may also send out an irregular process.

The balloons usually form on different sides of the cells successively and granules enter the balloons. The balloons in a m/4 KCl solution are very large, larger than in any other solution so far investigated.

The cell as a whole may move if the granuloplasm enters the balloon. If we observe at room temperature tissue in m/4 KCl solution soon after it has been taken from the ice chest, we usually find balloons and courts in the cells; these balloons change their position, are drawn in at one place and sent out at another. Granules enter these balloons and courts; but we usually do not find any circus movements which we

described in our previous paper. These circus movements consist in the rapid formation of a balloon; this balloon begins to move in a circle around a part of the cell circumference and simultaneously the granuloplasm pours as a whole into the balloon and follows the circus movement of the latter. Thus the whole cell, the hyaloplasm as well as the granuloplasm of the cell has become irregular in outline. Through the subsequent retraction of the balloon which presses upon the granuloplasm, both granuloplasm and hyaloplasm may again become rounded off and the cell take the appearance of a ball.

Such circus movements are, as stated, usually absent in cells kept at a lower temperature, but they appear and often in many cells simultaneously, as soon as the temperature of the specimen is raised, by placing the microscope near a lamp. Usually a cell performs those circus movements for some time; then the movement stops and the cell is at rest for a short time, when the movement begins again.

We thus see a distinct effect of temperature on the circus movements of balloons. In this regard they behave like other pseudopodial movements and in accordance with the effect of temperature on the rapidity of outgrowth. Also in m/4 KCl solution the outgrowth of the cellfibrin tissue is greater at incubator than at room temperature, or in the ice chest. Often the circus movements are only partial, the peripheral part of the granuloplasm carrying out these movements, while the central part remains stationary.

The first effect of warming the cells seems to be an increased Brownian movement of the granules within the cell; this is followed by the circus movements. In some cases the softening of the granuloplasm which is a prerequisite of the typical circus movement occurs without the formation of balloons. In such cases the granuloplasm may show a very active streaming within the cell without any subsequent outflowing of the granuloplasm, and without any circus movements following. In this case we have a condition analogous to protoplasmic movements within the cell such as is found in plant cells, unaccompanied by pseudopodial movement.

We have stated previously that in some cases even in a m/4 KCl solution some cells show the formation of tongue pseudopods. If we follow the fate of such cells, we notice that they usually continue to send out tongue pseudopodia, while the cells with balloons and circus movements continue to produce balloons, and circus movements. Exceptionally it may be seen that in a cell which presents a tongue pseudopod, a balloon with subsequent circus movements may develop.

These circus movements may continue in a specimen for several days. As we have stated they are only active at a higher temperature. However, if the temperature exceeds a certain optimum, the movements cease; thus while at the temperature of the incubator of about 34° circus movements may be seen at first, they cease here much earlier than in cells kept at a somewhat lower temperature.

The characteristic feature of the action of m/4 KCl is its effect on the granuloplasm. It shares the production of the balloons, half-moons, courts with hypotonic solutions of NaCl; but even in this case the action of m/4 KCl is much more marked than in a NaCl solution of equal osmotic pressure. In a m/4 KCl solution the balloons are much more frequent and often larger than in a m/4 NaCl solution. In addition a m/4 KCl solution softens the granuloplasm in such a way that it flows readily into the balloon, or in some cases moves actively within the cell and shows protoplasmic streaming. The mass of granules are apparently held together by a mass of denser protoplasm which at the periphery is perhaps so dense that it assumes the character of a second membrane. Under the influence of the m/4 KCl this membrane or mass of dense protoplasm is softened or partly dissolved and the granuloplasm flows very readily. Sometimes the softening or solution of the membrane may be localized and affect only a limited area of the membrane; then we see an opening in the periphery of the granuloplasm through which it streams into the balloon. At other times the softening is general.

We see then the action of m/4 KCl is essentially a softening, dissolving one. Not only is here the granuloplasm softened, but also the layer of exoplasm which forms the outer layer of the pseudopod. There is evidence which points to the conclusion that in a balloon this layer is softer than in a sharp tongue pseudopod, such as is found in isotonic or hypertonic solutions.

There are two components in this action of m/4 KCl. First, the softening effect of KCl, which is already indicated in isotonic solutions and secondly a weakly hypotonic effect which greatly increases the effect of the KCl but which in itself would be unable to produce the effects observed in a m/4 KCl solution.[1]

[1] We have found the effect of M/4 KCl identical in two different preparations of KCl, one a Kahlbaum preparation and the other a purified KCl preparation obtained from the laboratory of Doctor Denis at the Massachusetts General Hospital. It is therefore not probable that the effect of the solution was due to accidental admixtures.

In accordance with our previous observations we find that in solution of NH_4Cl the majority of the cells remain more or less unchanged as round or oval or slightly irregular granular cells; they appear almost as fixed cells; but in m/2 NH_4Cl some tongue pseudopodia and some drops and balloons may develop. In m/4 NH_4Cl the number of drops and balloons and of half-moons is greater; granules may pour into them through openings in the periphery of the granuloplasm; occasionally also a tongue pseudopod or a transitional pseudopod may be seen. There is very little active movement taking place in this solution, although there may be a layer of round granular cells on the upper as well as the lower surface of the fluid which surrounds the tissue. It is probable that at least many of these cells were passively detached from the piece, although some active movement may occur. Here also the cell movement is more considerable at higher than at lower temperature.

If we observe under the microscope near a lamp the tissue in a m/4 NH_4Cl solution soon after the specimen has been made, we notice some drops and balloous into which granules move. After one-half hour some larger balloons may be formed, some of which are directed toward the piece. Occasionally cells come out of the piece with tongue pseudopods, but outside of the tissue balloons are formed.

With $CaCl_2$ very few experiments were made. As in our previous experiments we found that m/2–m/4 $CaCl_2$ solutions produce a hyaline condition of the cells, which latter may be extended or contracted. They have glistening refractile protoplasm, which often includes a large vacuole; a few sharp pseudopods may form. The outgrowth in this solution is very slight.

In the literature I could find only some isolated observations, indicating that under certain conditions a change in the shape of a cell can be produced. Thus Zacharias observed that in a 5 per cent solution of Na phosphate certain spermatozoa and even an intestinal cell formed pseudopods and cilia (3). Verworn observed that if the water in which amoeba lived and moved was made faintly alkaline in the course of 15 to 20 minutes the character of the pseudopods changed to that characteristic of amoeba radiosa (4).

Effect of mixtures of salts on the outgrowth of cellfibrin tissue. If we remove the protein constituents of Limulus serum by boiling and filtering, a marked deterioration takes place; the outgrowth in the remaining fluid is very much diminished in the large majority of experiments. However, in certain cases the result is better, approaching even that in unchanged serum, although not quite reaching this level. As far as our

experiments indicate, this variation is due mainly to variations in the character of the cellfibrin used. If this is very resistant, the outgrowth may be relatively good. The same applies to sterile sea water. Here also the outgrowth is much less good than in serum and it may be lacking altogether, if the cellfibrin is not very resistant. The difference between serum and sea water under those conditions is very marked; but even with resistant cellfibrin the difference, while not so marked, is quite distinct.

Sea water in Woods Hole is alkaline in reaction. If we use instead a neutral mixture of salts, of similar composition to sea water (Van't Hoff solution) the results may become somewhat better, but so far the effect has not been so good as in serum even under the most favorable circumstances; the outgrowth has been less and the migrating cells have become hyaline at an earlier date than in serum. We find here again the same dependence upon the character of the cellfibrin used.

From this we may conclude that the proteins of the serum exert a protective influence upon the blood cells and at the same time, as we shall show elsewhere, that they are specifically adapted to the blood cells.

The effect of temperature on the rate of outgrowth. In a number of experiments we compared the rapidity of outgrowth in specimens kept in the ice chest (8°–10°), at room temperature (on the average 19°–24°) and in the incubator (about 34°). We found that the rate of outgrowth was more rapid at higher temperature; it was most rapid at the temperature of the incubator, least rapid in the ice chest. The difference between the rate of outgrowth at the different temperatures varies considerably in the individual experiments. Usually it was less than should have been expected according to the van't Hoff rule. This variability is due to variations in the size of the pieces, amount of fluid used and similar factors; but in addition there is another factor at work which tends to counteract the beneficial effects of the higher temperature. A rise in temperature accelerates not only those processes which lead to pseudopodial activity, but simultaneously it accelerates other processes in the cells which are unfavorable to pseudopodial activity, namely, a spreading out of the cells in contact with the glass, a process which is accelerated by a rise in temperature up to a certain limit. A further rise favors contraction (the exact temperature at which this change takes place remains still to be determined). Furthermore at a higher temperature there takes place a more rapid solution of granules and other changes which modify pseudopodial activity unfavorably.

In addition it is to be assumed that the larger number of cells which leave the cellfibrin tissue at the higher temperature, and spread out near the glass, present an obstacle to the subsequent outgrowth of other cells.

All these factors tend to counteract the favorable effects of a rise in temperature and thus the results are somewhat variable and usually less marked than should be expected according to the van't Hoff law.

The effect of temperature on the rate of outgrowth was noticeable not only in serum, but also in various salt solutions, even in m/2 KCl and m/4 KCl. the outgrowth being more considerable at the higher temperature.

Effect of temperature on the character of amoeboid movement: a. If we keep pieces taken from the same tissue surrounded by the same medium, in the ice chest, at room temperature and in the incubator (at about 33°–34°), we notice that the influence of the medium on the character of the pseudopods is noticeable in all the specimens irrespective of the temperature; the hypotonic effects of m/4–m/8 NaCl, the hypertonic effects of $\frac{3}{4}$ m NaCl, the influence of isotonic solutions and the characteristic effects of m/4 KCl solutions were distinct at low as well as high temperatures. There was however noticeable in many, although not in all experiments, some effects of a rise in temperature. In the first place the movements of hyaloplasm as well as of granuloplasm were more active at a higher temperature. The Brownian movement was more noticeable. We mentioned already that the circus movements of the cells suspended in m/4 KCl began only at a higher temperature. There was furthermore a tendency for the pseudopods to become broader, more rounded off at a higher temperature and to be sharper, thinner at a lower temperature. Thus the balloons, drops, courts and intermediate pseudopods seemed relatively more numerous at incubator than at room temperature, and still more so at ice chest temperature. This relative preponderance of the broader pseudopods at a higher temperature could be found in all kinds of solutions. This difference however was not observed in every case. Whether this effect was noticeable depended probably to some extent on the character of the tissue used. It will be necessary to carry out further experiments in order to make possible more definite statements as to the conditions under which this influence of temperature becomes noticeable.

b. There is however quite definite another effect of temperature which can be obtained regularly.

If we expose a specimen in a hollow slide to a higher temperature by placing it near an electric lamp in such a way that a thermometer being held in direct contact with the specimen reaches a temperature of 39° to 43° in about 3 to 12 minutes, and if we then remove the specimen from the direct effect of the lamp and place it for examination under the microscope we observe definite changes which have in common, *a*, the contraction of many blood cells and *b*, the production of droplets and small balloons.

If we heat a specimen in the manner indicated, we frequently observe under the microscope at first tongue pseudopodia forming in round granular contracted cells. Within 2 to 5 minutes this is followed by the formation of droplets or small balloons on the surface of the contracted cells. This continues for 10 or 15 minutes or even for a longer period. Isolated granules or the connected granuloplasm enter these droplets, and inasmuch as these droplets are often numerous, the filling of these droplets by granules may cause the appearance of mulberry forms. The unevenness of the cell outlines thus produced gradually disappears again and in the end, after 10 to 20 minutes, new tongue pseudopodia appear and subsequently the cell begins gradually once more to expand. We find thus a distinct cycle of changes as the result of the heating, first formation of tongue pseudopodia, then appearance of single or multiple drops and small balloons, and in the end again tongue pseudopodia.

However, in many cases this cycle is not complete. Sometimes we find at once the formation of drop pseudopodia to which are perhaps admixed a few tongue pseudopodia. These may persist throughout, or they may be followed in the latter stage by a mixture of drops and tongue pseudopodia in which the latter gradually become more prevalent, or may be the only ones visible in the last period. It is not quite clear what these differences depend upon. It is probable that tissues which in the unheated condition tend to the formation of balloon and drop pseudopodia and courts show after heating a similar tendency toward the formation of pure drops.

Insufficient heating is another cause which may lead to the formation of tongue instead of drop pseudopodia. Heating up to 36° to 39° within a period of 5 to 7 minutes often is not sufficient to cause the production of drops, but calls forth the formation of tongue pseudopods.

Again we find here an interchange between tongue and drop or balloon pseudopods. Thus drop or balloon pseudopodia may develop

spines and thus become tongue pseudopods. Or a tongue pseudopod may in its entirety or partially explode into a drop or balloon. The same cell may at one time show tongue pseudopods, later drops and then again tongue pseudopods; or a cell may show simultaneously at one place drops, at another place a tongue pseudopod. At a certain stage of the cycle there may appear successive balloons, which form at the same pole of the cell and into which the granuloplasm moves, thus insuring a locomotion of the cell as a whole.

These changes under the influence of heating occur in solutions differing as much as m/6 NaCl on the one hand and $\frac{3}{4}$ m NaCl on the other. They appear also in serum and in Van't Hoff solution. They develop quite readily in $\frac{3}{4}$ m NaCl solution; here either the complete cycle may appear or merely multiple drops. In all these media the drops are relatively small, but as far as can be judged without accurate measurements they are smaller in $\frac{3}{4}$ m NaCl than in isotonic or hypotonic solutions.

In m/4 KCl heating does not call forth the formation of typical small droplets, but the ordinary balloons may continue to be produced after heating. In heated cells at first no circus movements appear, but later they may again take place. Also in other solutions slight circus movements may appear in cells which have been heated previously. In m/4 KCl solution heating stimulates in some cases the formation of tongue pseudopods. In m/2 NH_4Cl a reaction is obtained with greater difficulty. In a number of cases heating does not call forth the production of tongue pseudopods or drops. In other cases some drops or tongue pseudopodia may be produced. In m/4 NH_4Cl likewise heating remains either without effect or it causes the formation of droplets and balloons. We see then that a certain reactive state of the protoplasm and a certain consistency is required in order to obtain these responses.

It is possible to obtain with the same specimen the same cycle repeatedly. After one cycle has been finished the specimen may be heated again and a new response is called forth. We obtained this reaction as often as 5 or 6 times in succession with the same specimen.

If we put the specimen later into the ice chest for several hours, the cells begin to expand again in their usual manner, and through heating again the typical reaction may be obtained. However, the return to the lower temperature does not necessarily mean a complete return to the old condition of the protoplasm. The tissue has to some extent been definitely changed through the previous heating and in the ice chest certain cells continue to produce balloons, drops or courts, although

tongue pseudopodia may also appear. A moderate heating of such tissue under the microscope near the lamp may again temporarily stimulate the production of tongue pseudopodia.

Differences in the amoeboid activity of different cellfibrin tissues. As we have mentioned previously, the cells migrating from different kinds of cellfibrin of Limulus behave in certain cases differently, while in other cases the cellfibrins are equal. Usually fresh, 1- to 2-day old tissue is better than 5-day old tissue, and the latter is superior to 10-day old fibrin. But this is not always the case; other factors besides age play a part in determining the behavior of the tissue. Thus cellfibrin which has partly retracted after standing for 5 days or longer in the ice chest was found inferior even if parts of the tissue were used which had not yet retracted. In such cases the tendency to retraction is present even in such parts of the tissue in which the change is not yet visible. The elastic tension under which the tissue is held has increased. Usually such tissue is not equal to fresh cellfibrin.

The state of health of the Limulus from which the experimental tissue was obtained is of special significance. If an anemic individual served for this purpose, the cellfibrin is less active and resistant, and younger tissue from an anemic, weak individual may be inferior to the somewhat older tissue from a healthy Limulus. On the other hand I did not find a noteworthy difference between tissue prepared from blood which gave a deeply blue color after standing and other tissues obtained from somewhat paler blood.

The differences between various tissues may be as follows: 1, The more resistant healthier and younger tissue shows usually a better outgrowth. *2,* In such tissue the cells have usually a greater tendency to preserve the granules than in inferior tissue, where in some cases the cells become hyaline more rapidly. *3,* The character of the pseudopods differs in both cases. In inferior tissues there is on the whole a greater tendency to the formation of balloon and drop pseudopods, while in very healthy young tissues the tendency is greater toward the formation of tongue pseudopodia. Of course the character of the media in which the tissue is placed determines how far that tendency is realized; but in hypertonic ($\frac{3}{4}$ m NaCl) as well as in hypotonic solutions (m/6 NaCl) this tendency finds expression.

The degree of variation caused by differences in the experimental tissue varies greatly in different cases and may also vary in different solutions. While in certain experiments the difference between different tissues may be relatively slight in favorable media such as healthy

Limulus serum unheated or heated to 56°, 60° or 70° or m/2 NaCl it is very much more pronounced if we use more unfavorable media, as, e.g., Limulus serum heated to 100°, very old Limulus serum, heterologous serum, seawater or even Van't Hoff solution. In such cases there may be a considerable outgrowth if good tissues are employed, while the outgrowth is very slight or lacking altogether if the inferior tissue is used.

The factors which determine the direction of movement of cells from the cellfibrin tissue into the surrounding medium. As in the case of other tissues, the constituent cells of which are motile, we can also in the case of the experimental Limulus tissue recognize two factors which determine the direction of outgrowth, namely, *a*, The stereotropic response which induces tissue cells to move in contact with a solid base (this reaction was first described by us in 1898 and later by Harrison and others); and *b*, the tendency to centrifugal growth.

The former we explained on the basis of our observations in amoebocytes as due to changes in consistency in the surface layer of the cells which make them sticky. We pointed out the relationship between this phenomenon and other phenomena, like phagocytosis, the formation of foreign body giant cells, and the giving off of substances accelerating the coagulation of the blood on the part of blood cells. In all these cases the contact with a solid body, strange to the life of the normal cell, leads to the response, which apparently everywhere consists in a diminution in the consistency of the cell surface. In some cases this leads to an agglutination of cells, such as we find in the formation of experimental cellfibrin tissue; in other cases the diminution in consistency of the cell surface proceeds so far that two cells which at first stick together become united into one. This may take place in the formation of foreign body giant cells and in the formation of other kinds of syncytia.

The tendency to centrifugal growth leads to a radial outgrowth which we notice in the growth of various tissue elements and which is especially noticeable if tissues grow in the test tube. In previous papers we considered the possibility that differences in electric potential between the side of injury and the normal side of the tissue might direct the outward growth of the cells. However, some preliminary experiments which we carried out in the summer of 1919 and of 1920 (the latter were done in association with Mr. Samuel E. Pond) and in which we exposed small pieces of cellfibrin tissue in a medium of Limulus serum to the action of a constant galvanic current, so far failed to show any influence of the current on the migration of the cells.

In addition we carried out several experiments in which we exposed in a dark room the experimental tissue kept as usual in hollow slides to the beam of an electric lamp. In each experiment a number of slides were placed in the field of the light rays, control slides were kept in the dark. The intensity of the light which was used varied approximately between 2500 and 1000 meter candles.[2]

The time of exposure varied approximately between 4 and 7 hours in different experiments. The tissues were suspended in serum, in m/2 NaCl and in m/4 NaCl. There was no noticeable difference between the outgrowth on the side directed toward or away from the light, neither was there a marked difference between these specimens and control specimens kept in the dark. Neither the direction nor the intensity of the outgrowth was consistently influenced by the light.

In other experiments we found that if we place two pieces of tissue near each other, either in a dish or on a coverglass in a hollow slide, the tissues do not exert a repellent effect upon each other; the cells coming from each piece grow toward each other. The same result we obtained in experiments in which we placed two pieces of mammalian tissue in the test tube; both pieces were surrounded by coagulum of blood plasma. The cells coming from the two pieces were not repelled by each other. If we observe the direction in which the pseudopods are sent out we notice that in the cells which emigrate from the piece of tissue the pseudopods are directed outward; this applies also to the balloon and drop pseudopods which form in certain media. In cells which have moved away from the tissue the majority of the pseudopods are likewise usually directed outward, but others point in the opposite direction, namely, toward the piece, or they are sent out in a lateral direction. Cells may move toward each other and meet and stick together. Subsequently the agglutinating cells send out pseudopods in such a way that the cells become again separated from each other.

On the basis of our observations and experiments the centrifugal outgrowth may be interpreted by assuming that contact with a cell leads to a resting condition at the place of contact and that the strange environment surrounding the cells elsewhere produces changes in consistency of the surface of the cell and leads to the production of pseudopods. This would insure a tendency of the cells to move away from each other. After the cells have once left the piece of tissue their centrifugal growth is to some extent a chance phenomenon. They have a

[2] I wish to express my thanks to Dr. Selig Hecht who put his apparatus at my disposal for these experiments.

much better chance to move without finding an obstacle in migrating in the direction away from the tissue; in moving toward the tissue they meet much more frequently other cells to which they stick and from which they become separated only by the sending out of pseudopods in an outward direction. Thus according to the law of chance the tissue would spread out more and more in a centrifugal direction.

It is furthermore probable that there exists a tendency for a cell to persevere in the sending out of pseudopodia at a certain pole. We often notice that tongue as well as balloon pseudopodia are sent out consecutively at the same pole. At other times, however, the direction changes; this is especially frequent in the case of the rapidly forming pseudopods, the balloons and drops. But it occurs also in the case of tongue pseudopods. Pseudopods can be sent out at any part of the cell surface; but we noticed that if a drop pseudopod had developed at one pole and this drop had been subsequently filled out by granules, the opposite pole was favored for the formation of tongue pseudopods, while the place where the drop had formed remained at rest at least for some time afterward.

If we consider all these factors we come to the conclusion that chance plays an important part in the centrifugal growth of the tissue.

The Limulus clot considered as a tissue. Further light is thrown on the character of the pseudopodia and of amoeboid movement of the amoebocytes by a consideration of the blood clot of Limulus.

In my earlier papers on the blood of arthropods I pointed out that the blood clot of Limulus represents a tissue which is free from fibrin. I showed that under certain conditions structures can be obtained which resemble connective tissue (in isotonic solutions, as a result of mechanical factors), under other conditions structures are produced which resemble epithelial tissues (in hypotonic, slightly alkaline solutions). Last summer I observed that by the use of slightly hypertonic solutions structures can be obtained under certain conditions which closely resemble nerve and glia tissue. While of course no identity exists between such tissues and the experimental structures, yet I believe that these analogies are of significance and indicate conditions under which the normal tissues develop. They point to the conclusion that differences in the consistency of the various cells which constitute these tissues play a part and that these differences in consistency are to some extent analogous to those which lead to the experimental production of these structures in vitro. I believe furthermore that in the normal tissue formation changes in the consistency of the surface layer of the cells

which lead to agglutination play a similar rôle to that found in experimental tissue and that the sending out of amoeboid processes and movements of granules into the pseudopods are of significance in both cases. Also the effect of pull and pressure is similar in the case of the normal tissues and of Limulus clot.

In 1919 we showed that the essential processes of wound healing can be imitated in tissue which has been prepared experimentally in such a way that an even layer of agglutinated cells is produced.

There is however a special reason why the resemblance of the Limulus clot to a tissue should be discussed in this connection. The typical Limulus tissue is a solid structure, which resembles in this respect fibrin and connective tissue. It resembles also fibrin and connective tissue in that it retracts spontaneously and especially if cuts are made into the tissue. Now this elasticity is dependent upon a change in the physical character of the cells after they have begun to spread out or after a part of the cell protoplasm has been drawn out or flown out from ruptured cells. These structures acquire elasticity and their outer layer becomes gelatinous. This applies especially to the pseudopods which are sent out by the cells. We have thus an additional proof of the change in consistency of the cell protoplasm during amoeboid movement; but while the changes in consistency during amoeboid movement are reversible, in tissue formation we have to deal with furthergoing changes which are no longer completely reversible. However, some cells which are widely spread out, and connected with other cells by means of long pseudopodia and form thus a part of a tissue can in certain cases again be made to contract by exposure to a higher temperature.

We see therefore that the cell protoplasm after it has spread out acquires certain new physical properties which cause it to contract. It is conceivable that an analogous change in physical properties is also at the base of the contractive phase in amoeboid movement. The latter would represent an acute process, while the tissue retraction would represent the same phenomenon as a slow, non-reversible process.

In previous papers I showed that in Limulus blood no conversion of fibrinogen into fibrin takes place; a real coagulation does not occur here, but merely an agglutination of blood cells (5). This coagulum is therefore comparable to a tissue. Last summer I found additional facts which confirm this conclusion. We could show that in all those arthropods in which a true coagulation takes place, I did not succeed in preparing an experimental cellfibrin tissue by the methods employed

in the case of Limulus blood. The fibrin which surrounds the clumps of agglutinated blood cells prevents the outgrowth of the amoebocytes. The clot of lobster, bluecrab, spidercrab, rockcrab blood cannot be used as experimental tissue.

That our interpretation of this phenomenon is correct follows also from the fact that if we surround a piece of Limulus cell fibrin with a layer of uncoagulated plasma of lobster, bluecrab or rockcrab blood, the surrounding clot prevents the outgrowth of the cells. While the serum obtained from the corresponding clot permits an outgrowth, the amoebocytes do evidently not develop a sufficient kinetic energy to overcome the mechanical obstacles presented by the fibrin.

We may therefore conclude that in the blood clot of Limulus such an envelope of true fibrin is absent.

On the character of amoeboid movement and tissue formation. In 1901 we observed that the sending out of pseudopods in amoebocytes depended upon a change in consistency of the protoplasm of the cells. During the process of formation the cytoplasm was soft and very soon afterward the outer part of it became solid (6). Our subsequent observations confirmed the solid character of the pseudopods (7) and especially Jennings noticed the solid character of pseudopods also in amoebae (8).

We interpreted therefore in our earlier paper amoeboid movement as due to changes in consistency of the protoplasm comparable from a mechanical point of view to the transformation of fibrinogen into fibrin, but a reversible process. The recent studies of Pauly and especially Jacques Loeb on proteins make understandable such reversible changes which take place whenever the neutral protein changes either through combination with acid or alkali or even with a neutral salt into a protein salt. Under those conditions the protein takes up H_2O and changes in viscosity take place. On this basis we explained previously the changes in stickiness and in shape which occur in the blood cells as soon as they leave the body of the animal. In agreement with this interpretation are the marked changes which we observed in the physical properties of the blood cells under various conditions. These changes in consistency and elasticity were tested by us directly by exerting pressure and traction upon the blood cells and by rolling them up between glass slides (9).

Secondarily added to these changes in consistency are changes in surface tension which take place whenever the changes in consistency lead to a sufficient liquefaction. These we noted in certain hypotonic solutions, but particularly in slightly hypotonic KCl solutions. The

balloons and drop pseudopodia, the courts and half-moons show very clearly the influence of surface tension.

In this paper we have shown that we can experimentally change the consistency of the protoplasm and that the character of the pseudopodia corresponds to the consistency. This is a further proof in favor of the conclusion that the formation of pseudopods depends upon changes in consistency in the protoplasm. These changes in consistency not only modified the character of the pseudopods but the whole cell and a very viscid consistency of the protoplasm is associated with pseudopods whose character agrees with such a viscid condition. The same correspondence we find between a soft, liquid protoplasm and the character of the pseudopods. We can experimentally grade the consistency and accordingly the pseudopodia. The size and distance of the granules, their fixedness or their ability to carry out Brownian movements, the character of the movement of the granuloplasm, whether it moves slowly, connectedly as a whole or rapidly and as individual granules, the size of the cell, indicate the character of the protoplasm.

As we have stated the number of amoebocytes which show the typical response to the changed environment varies in different cases. Not all the cells present the typical pseudopodia in hypotonic or hypertonic solutions. Certain cells are evidently more resistant to the osmotic changes in the environment. In addition, as we have stated previously, it is probable that especially in hypotonic solutions gradually an increase in osmotic pressure occurs through solution of certain cell constituents. These changes in the medium in which the cells are placed not only modify the character of the cytoplasm, but also the rigidity of the pseudopodial exoplasm. While normally it resembles solid gelatine, it is somewhat softer in the balloon pseudopods. Here it adapts itself to the internal pressure of the fluid and can readily be pushed in through pressure from the outside. It appears therefore that the degree of solidification of the external protoplasm may also vary in intensity. We saw that all possible transitions exist between the typical tongue pseudopodia and the balloon and drop pseudopods; furthermore one kind can be transformed into the other kind and the same cell can produce in succession first one, then the other kind. Moreover we saw that the cell as a whole, particularly the granuloplasm, behaves toward these drop and balloon as it does toward the other pseudopods—it moves into them.

As we have stated in our previous papers the majority of the granules in the amoebocytes of Limulus are situated near the surface of the cell.

Preceding amoeboid movement the consistency of the exoplasm becomes more fluid. This change is strictly localized and leads to the formation of a sharp spine or sharp tongue; this is often followed by a widening of the area of softening and thus a somewhat broader tongue is formed at the base of the spine and lifts the former up. This is the most frequent mode of origin of the typical tongue pseudopodia. In other cases, however, a pseudopod starts as a broader, rounded-off pseudopod and secondarily develops on top a sharp ridge or spine.

While this process of partial liquefaction is taking place and the softened hyaloplasm flows out assuming a shape in accordance with the degree of viscosity of the substance, and in certain cases in accordance with the amount of and the degree of fluidity of outflowing endoplasm, the granules underneath the softened area seem to be held together by a more viscous material. Gradually a pull is exerted on this granuloplasm and it also moves into the pseudopod. This is perhaps accompanied by a process of partial liquefaction in this material surrounding the granules. Sometimes it appears as if a sharp opening were produced through liquefaction in a shell separating the granuloplasm from the pseudopod and this opening permitted now the granules to pour into the pseudopods. This is observed especially under conditions under which the consistency of the whole cell has been diminished. It is therefore very probable that changes in consistency occur not only in the exoplasm, but also in the granuloplasm. Especially marked is this softening of the granuloplasm under conditions which lead to the formation of circus movements.

The formation of the pseudopods depends then upon processes of liquefaction and upon a separation of the more liquid and the more solid parts of the cell, and in this respect the formation of pseudopods, the formation of droplets in cytolysis and the formation of a fertilization membrane in the mature ovum are related phenomena. We have produced experimentally in the amoebocytes analogous processes. While the mode of formation of the fertilization membrane and of the corresponding structures in the amoebocytes is not identical, the principle underlying the formation of both structures is the same. The typical formation of pseudopods represents a change of medium intensity while the formation of the structures resembling eggs with fertilization membranes on the one hand and long ramified threads on the other represents extreme processes in a continuous series.

As we have seen, it is possible to change the character of the amoeboid movement and of the pseudopods, not only through a change in

the medium in which the blood cells are suspended, but also through changes in the temperature of the medium. We saw that a rise in temperature above that of the room favors a contraction of the blood cells and that even cells which in contact with the glass had spread out can again contract. If the rise in temperature is still greater, the cycle of changes takes place which we have described above; prominent among these changes is the temporary formation of multiple drop pseudopodia and mulberry cells.

In addition it is at least probable that a higher temperature in general favors the broadening of pseudopods. The most probable interpretation of these phenomena is that it is due to a greater liquefaction of the protoplasm produced by the increase in temperature. If the liquefaction (as indicated by the Brownian movement of the formerly fixed granules, which is sometimes noticeable) is sufficiently marked, the cell rounds off, loses its stickiness, becomes partially or entirely detached from the slide and sends out drop instead of tongue pseudopodia. This change is to some extent reversible; the cell protoplasm becomes again more consistent and sticky and spreads out, if returned to the ice chest. However, as we have seen, a permanent change in consistency of the protoplasm has been produced through this heating. Changes which take place spontaneously but slowly have been thus accelerated.

With this interpretation would agree the observations of Greeley who found that a lowering of the temperature causes a solidification of the protoplasm, while raising the temperature has the opposite effect and favors the taking up of water (11). K. Gruber observed in amoeba proteins that exposing the organisms to a temperature of 30° for several hours causes the formation of a peripheral hyaline layer which Gruber interprets as due to liquefaction; returned to cool water, hyaline drop-like processes formed, which gave the amoeba the appearance of a mulberry; this is subsequently followed by a resumption of the normal movements (12). There is apparently some similarity between these processes and those observed by us in blood cells of Limulus after heating. The rise in temperature sets in motion a cycle of changes associated with an increase in the fluidity of the cell.

It is very probable that the droplike formations which A. Golubew (13) and R. Klemensiewicz (14) produced in the leucocytes of amphibian blood through the application of interrupted electric currents are of a similar nature. Strong stimuli of various kinds would then produce similar changes in consistency of the protoplasm and similar reactions.

We conclude then in accordance with our previous interpretation that changes in consistency of the protoplasm are the primary factors in the amoeboid movement of amoebocytes and of the leucocytes in general, and that changes in surface tension may follow secondarily. That it holds good also in the case of amoebae is made probable through the observations of Jennings (15), Dellinger (16), E. Schultz (17), Kite (18), Mast (19), Chambers (20) and Hyman (21). Even Rhumbler who following Berthold, Quincke and Verworn maintains that in general amoebae are liquid and that the amoeboid movement is primarily due to changes in surface tension in a liquid medium, has admitted that in certain cases changes in consistency of the protoplasm play a part (22). We believe the evidence points to the conclusion that in general changes in consistency are the primary factor in amoeboid movement, that surface tension changes follow secondarily and that only if the liquefaction has proceeded sufficiently far may perhaps surface tension changes represent the primary factor.

Our observations on the conditions which modify amoeboid movements have a bearing also on the analysis of tissue formation as we attempted it on the basis of our experiments. The stickiness which develops in the isolated cells as a result of mechanical and chemical stimuli, and which leads to agglutination and is thus at the base of the tissue formation, cannot be caused primarily by changes in surface tension in a liquid substance, but must be due to changes in consistency similar in character to those taking place in gelatine when it becomes sticky. These changes are probably dependent upon changes in the relation between proteid molecules and the surrounding water. Closely related to these changes are those upon which depends the stereotropism of tissue cells, phagocytosis and the formation of foreign body giant cells, as we have shown on previous occasions.

The stereotropic reaction consists in a spreading out of the cell and the changes associated with this process lead to a creation of potential energy. The spread-out cells and cell fibers are held under tension and retract when cut. In the course of their formation elasticity has developed. This is probably also the cause of the retractibility and tension which we find in various natural tissues. These are likewise due to these changes in consistency of the protoplasm.

SUMMARY

1. The amount of outgrowth from the amoebocyte tissue depends upon the osmotic pressure of the medium. The optimal point is at a concentration of about m/2 NaCl. From there a decrease takes place toward both sides, the decrease increasing with diminishing and increasing osmotic pressure.

2. The factors on which the behavior of amoebocytes emigrating from (experimental) cellfibrin tissue (rapidity of movement, degree of the stereotropic spreading out, preservation of granules, character of pseudopodia and amoeboid movement, consistency of the protoplasm) depends are the following: *a*, character and age of the tissue used; *b*, osmotic pressure and character of substances in the medium surrounding the tissue; *c*, temperature; *d*, time during which the tissue is exposed to the medium. It is possible to modify experimentally the majority of these factors and thus to modify the character of the pseudopods and of the amoeboid movement.

3. Through changes in osmotic pressure of the surrounding medium it is possible to change the consistency of the cell and concomitantly the character of the amoeboid movements.

4. K, NH_4 and Ca have typical effects on the cells and their amoeboid movements. KCl in a slightly hypotonic solution leads to a marked softening of the whole cell, including the granuloplasm and thus it is possible to produce circus movements of the cell and occasionally intracellular plasmastreaming. These movements do not usually occur at a temperature of 10°; they are accelerated by a moderate rise in temperature (to about 25°–28°).

5. Mixtures of salts, similar to those found in the blood have so far not given results as good as Limulus serum. They are especially inferior to serum, if cellfibrin is used which is less resistant.

6. A rise in temperature increases the rate of outgrowth from the cellfibrin tissue. This outgrowth however is usually less marked than should be expected according to the Van't Hoff rule. This is probably due to the fact that a rise in temperature favors at the same time destructive changes in the cells.

A rise in temperature also increases the protoplasmic movements within the cell. Under certain conditions it leads to a broadening and rounding off of the pseudopods. If the rise in temperature is sufficiently sharp a cycle of changes can be produced, the most typical part of

which is the formation of multiple drop pseudopodia and of mulberry forms. Although these changes are to some extent reversible, heating causes a permanent alteration of the protoplasm.

7. The cells derived from different cellfibrin tissues differ in their behavior. Older tissues and tissue obtained from anemic animals manifest their inferiority especially in less favorable media.

8. The migration of cells from tissues is determined by two factors: *a*, the stereotropic reaction, and *b*, the tendency toward centrifugal growth. The former depends upon changes in consistency of the protoplasm induced by changes in environment and especially by contact with solid bodies. As we have pointed out previously, the same reaction plays a part in phagocytosis, in the formation of foreign body giant cells (where the foreign body may produce still further-going changes in the cell consistency) and in the giving off of substances accelerating the coagulation of the blood. The tendency toward centrifugal growth can best be explained by assuming that contact with a cell leads to a resting condition at the place of contact, while the change in environment elsewhere leads to movement. To some extent the centrifugal growth would thus be a chance of phenomenon. So far we have not found either a galvanotropic or a phototropic reaction in the amoebocytes.

9. Additional proof is given that the clot of Limulus blood is not true fibrin but consists merely of agglutinated cells. It is shown that this clot can be considered as the type of a tissue and that the factors underlying the formation of this clot are similar to those active in tissue formation; prominent among those factors are changes in the consistency of the protoplasm. Gradations in the consistency of the protoplasm lead to the production of various structures which are analogous to various normal tissues.

10. It is probable that amoeboid movement in blood and tissue cells as well as in protozoa, the agglutinative processes underlying the formation of normal and experimental tissue are due to primary changes in consistency to which may be added secondarily changes in surface tension. It is furthermore probable that the formation of pseudopods and the processes leading to the formation of a fertilization membrane in the mature ovum are related phenomena.

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A STUDY OF THE DISTRIBUTION OF IODINE BETWEEN CELLS AND COLLOID IN THE THYROID GLAND

III. The Effect of Stimulation of the Vago-Sympathetic Nerve on the Distribution and Concentration of Iodine in the Dog's Thyroid Gland

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For many years it has been held that the thyroid gland is supplied with true secretory nerves. In support of this assertion there is considerable anatomical evidence and some physiological evidence. Of late the nerves which anatomists have traced into the thyroid gland and have considered to be possibly secretory in function have been declared to be branches of the cervical sympathetic nerve. And recent physiological work has tended to confirm this view. Only twelve years ago Wiener (1) published the report of experiments from which he concluded that extirpation of the inferior cervical ganglion produces a marked atrophy of the thyroid gland on the side of the extirpation. Wiener maintained that no comparable effect on the lobe of the thyroid gland on the side of the operation could be produced by vagotomy or by removal of the superior cervical ganglion. More recently Rahe et al. (2) announced that they were able to produce a quite marked diminution in the iodine concentration of the lobe of the thyroid gland on one side by stimulating the thyroid nerves in several different ways. They stimulated the nerves of the superior thyroid artery, the intact vago-sympathetic nerve as well as the vago-sympathetic nerve near the level of the superior cervical ganglion after ligating the nerve low in the neck and cutting the nerve central to the point of stimulation. They found that the most marked loss was brought about by the stimulation of the intact vago-sympathetic nerve.

Watts (3) undertook to find out whether or not the results obtained by Rahe, Rogers, Fawcett and Beebe might be due to vasomotor

changes in the gland on the stimulated side. Watts likewise found that he could reduce the iodine content of the right or left lobe of the thyroid gland of the dog by stimulating the "cervical sympathetic isolated from the vagus sheath" and the "nerve filaments accompanying the superior thyroid vessels." However Watts maintained that he could cause some diminution in the iodine content simply by periodically reducing the blood flow through the gland by "occluding the main thyroid artery" the nerves of which had been dissected away. Hence he concluded that all of the effects of stimulation on the iodine content can be accounted for by the coincident vasomotor changes which he showed to be present.

Positive evidence of the secretory effect of sympathetic stimulation has been reported by Cannon and his co-workers (4) in several communications. Working with cats they sutured the phrenic nerve with the cervical sympathetic nerve and observed following the operation increased basal metabolism, respiratory hippus and falling hair which they interpreted as the results of hypersecretion of the thyroid gland caused by the periodic bombardment of the gland by impulses carried from the respiratory center to the gland's secretory nerves. Cannon and Cattell (5) adduced additional evidence as to the rôle of the sympathetic nerves in experiments dealing with the electrical condition of the gland. Following the stimulation of the upper thoracic sympathetic nerves or the injection of epinephrin they were able to show a definite action current in the thyroid gland after a latent period of five to seven seconds. Recently Cannon and Smith (6) maintained that gentle massage of the thyroid gland or stimulation of the cervical sympathetic nerve increases the rate of the denervated heart. The denervated heart is said not to be affected when the cervical sympathetic nerve is stimulated after removal of the thyroid gland. Moreover Levy (7) observed that the pressor effect of epinephrin after a variable latent period is increased by the stimulation of the cervical sympathetic nerve. He declared that stimulation of the cervical sympathetic nerve has no such effect after thyroidectomy.

However the conclusions based on the experiments mentioned above have not been universally accepted. Burget (8) was unable to alter the thyroid gland noticeably either by uniting the phrenic and cervical sympathetic nerves or by removing a section of the cervical sympathetic nerve. Marine, Rogoff and Stewart (9) sutured together the phrenic and cervical sympathetic nerves in several cats. They demonstrated a functional union between the phrenic and cervical sympathetic nerves

but observed no exophthalmos, tachycardia or respiratory hippus in their animals. There was no apparent difference either grossly or histologically in the lobes of the thyroid gland on the operated and the non-operated sides. Troell (10) reported that he was unable to produce either exophthalmos or respiratory hippus by suturing the proximal end of the phrenic nerve to the cervical sympathetic nerve. Employing cocaine as a sensitizer for sympathetic nerve endings, Mills (11) did not observe, following the repeated injection of cocaine, any alteration in the amount or nature of the thyroid secretion as judged by what is known of the gland's histology. Finally Rogoff (12) records one experiment in which he drew blood from a vein of the left lobe of the thyroid gland and at the same time stimulated the cervical sympathetic nerve on that side in the hope of increasing the secretory activity of the stimulated lobe. From the right lobe he also collected blood by way of a vein. While drawing the blood he massaged the right lobe to some extent but did not stimulate the right cervical sympathetic nerve. He found that specimens of dried blood from each lobe were potent when fed to tadpoles. But he could detect iodine chemically only in the blood from the non-stimulated lobe. Moreover the non-stimulated lobe had a lower iodine content than the stimulated lobe.

In connection with some studies on the distribution of iodine in cells and colloid in the thyroid gland I attempted to alter acutely the total iodine content of the gland by stimulating the vago-sympathetic nerve of the dog. Some inconsistencies in the results in the early part of the work forced me to investigate the matter more carefully and to repeat the work of Rahe et al. (2) and of Watts (3).

Methods. Dogs were used in all of the experiments. All except those whose numbers are above that of no. 78 were given daily feedings of iodine over a period of one to eleven days. The daily feeding consisted of a capsule containing two drops of tincture of iodine in starch. In the animals fed iodine the stimulation of the vago-sympathetic nerve was undertaken from two to ten days after the last feeding of iodine. Throughout the experiments the animals were *lightly* anesthetized with ether. Platinum wire electrodes were applied to opposite sides of the carefully isolated vago-sympathetic nerve and shielded from all surrounding tissues by glass. In all of the experiments except those recorded in table 5, a tetanizing current from three to six times as strong as that sufficient to bring about a pupillary dilatation and apparent protrusion of the bulbus oculi was employed. The regulation of the strength of the current was made possible by a rheostat inserted in the

circuit. Again in all of the experiments except those to be found in table 5 the stimulating current throughout the period of stimulation was made for about 0.8 second at intervals of 1.6 seconds by means of a clock and ratchet device. The strength of the current used and the rate at which the current was made in the experiments of table 5 are described below.

TABLE 1

Variations in the iodine content of neighboring specimens of the same lobe of the thyroid gland

ANIMAL NUMBER	NUMBER OF FEEDINGS OF IODINE	LOBE OF THYROID GLAND	WEIGHT OF SAMPLE OF DRIED GLAND ANALYZED	IODINE IN DRIED GLAND ANALYZED	DIFFERENCES IN THE CONCENTRATION OF IODINE IN NEIGHBORING SPECIMENS OF THE SAME LOBE
			gram	*per cent*	*per cent*
68	11	Left	0.1192	0.331	0.093
			0.1378	0.424	
		Right	0.1525	0.418	0.037
			0.1668	0.455	
74	6	Left	0.6931	0.366	0.005
			0.6420	0.371	0.034
			0.6846	0.405	0.039
		Right	0.4549	0.370	0.006
			0.4931	0.364	0.019
			0.5533	0.383	0.013
88	0	Left	0.4454	0.121	0.011
			0.5298	0.132	
		Right	0.7857	0.119	0.010
			0.4528	0.129	
105	0	Left	0.7241	0.158	0.018
			0.7138	0.176	
		Right	0.4489	0.163	0.015
			0.4516	0.148	

After a number of experiments had been performed it was found that there is considerable variation in the iodine content of adjoining pieces of the *same* gland. In table 1, for example, are given a few analyses of neighboring specimens of the same gland. The differences in neighboring portions of the glands are somewhat greater in iodine-fed animals.

TABLE 2

Quantitative determination of iodine in whole gland and in cells free from colloid material of thyroid glands of dogs whose isolated vago-sympathetic nerve had previously been stimulated for approximately three hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	WEIGHT OF WHOLE GLAND USED	IODINE IN WHOLE GLAND	WEIGHT OF CELL MASS USED	IODINE IN CELL MASS	RATIO OF PER CENT OF IODINE IN CELLS TO PER CENT OF IODINE IN WHOLE GLAND	APPARENT GAIN (+) OR LOSS (−) IN CONCENTRATION OF IODINE IN WHOLE GLAND OF STIMULATED LOBE
			gram	*per cent*	*gram*	*per cent*		*per cent*
42	Left	2 hrs. 15 min.	L 0.1792	0.531	0.2533	0.371	0.699	−0.029
			R 0.1693	0.560	0.1360	0.303	0.541	
45	Left	3 hrs. 0 min.	L 0.4602	0.148	0.5117	0.011	0.074	+0.046
			R 0.5336	0.102	0.4750	0.014	0.137	
46	Left	3 hrs. 30 min.	L 0.4982	0.098	0.3445	0.014	0.143	−0.006
			R 0.5252	0.104	0.2284	0.017	0.163	
54	Right	2 hrs. 30 min.	L 0.3964	0.177	0.2713	0.036	0.203	−0.030
			R 0.4241	0.147	0.3883	0.019	0.129	
55	Right	2 hrs. 40 min.	L 0.6880	0.232	0.2700	0.031	0.134	−0.046
			R 0.5509	0.186	0.2280	0.046	0.247	
56	Right	2 hrs. 10 min.	L 0.4536	0.121	0.3683	0.016	0.132	−0.012
			R 0.4795	0.109	0.3059	0.014	0.128	
62	Left	3 hrs. 5 min.	L 0.0836	0.494	0.0480	0.078	0.158	−0.029
			R 0.0558	0.523	0.0612	0.082	0.157	
64	Right	3 hrs. 50 min.	L 0.0892	0.110	0.1019	0.008	0.073	+0.017
			R 0.0692	0.127	0.1190	0.010	0.079	
65	Right	3 hrs. 0 min.	L 0.1179	0.436	0.1086	0.037	0.085	+0.030
			R 0.1419	0.466	0.1064	0.052	0.112	
66	Right	3 hrs. 0 min.	L 0.0709	0.485	0.0733	0.041	0.085	+0.008
			R 0.0593	0.493	0.0783	0.038	0.077	
67	Left	3 hrs. 0 min.	L 0.1373	0.464	0.1842	0.041	0.088	+0.013
			R 0.1237	0.451	0.1613	0.040	0.089	
68	Left	2 hrs. 45 min.	L 0.2570	0.377	0.1703	0.031	0.082	−0.060
			R 0.3193	0.437	0.1379	0.028	0.064	
69	Left	3 hrs. 0 min.	L 0.5360	0.605	0.2627	0.083	0.137	+0.036
			R 0.3957	0.569	0.1476	0.092	0.162	
71	Right	3 hrs. 0 min.	L 0.3651	0.346	0.1718	0.026	0.075	−0.041
			R 0.2990	0.305	0.1310	0.029	0.095	

Hence it was thought desirable to analyze not single blocks or samples of dried powdered mixtures of whole gland but to analyze the *whole* gland in each case. In experiments in which this last mentioned technique was employed the whole gland was carefully cleaned of connective tissue and blood vessels, and thoroughly dried first over an electric hot plate and then in an electric oven. After two to three hours' drying in the electric oven the gland was broken into several pieces whose weight was about 0.5 gram each and whose number therefore depended on the size of the gland. The iodine determinations were made according to the method earlier described by Kendall (13). By analysis of powdered thyroid of known iodine content the accuracy of the method (to about 0.008 mgm. of iodine) and the purity of the reagents used were frequently examined and found to be satisfactory.

The strength of the current employed in all of the experiments recorded in table 2 was three times that sufficent to cause dilatation of the pupil and apparent protrusion of the bulbus oculi. Both the stimulated and the non-stimulated vago-sympathetic nerves were cut in two places: at a point in the neighborhood of the eighteenth tracheal ring and also at a level a little above that of the hyoid bone. The reason for cutting the non-stimulated nerve in such a manner was to eliminate the possible effect of tonic secretory impulses on the non-stimulated lobe. The vago-sympathetic nerve was stimulated a little above the point at which it was cut low in the neck. In the above experiments the ratio of the percentage of iodine in cells to the percentage of iodine in whole gland was determined by a method previously described (14). From the data given in table 2 it may be seen that stimulation of the vago-sympathetic nerve under the conditions described is without appreciable effect on either the ratio value or the concentration of iodine in the whole gland.

When it was found that there was no consistent diminution in the concentration of iodine in the stimulated lobe only the stimulated vago-sympathetic nerve was sectioned in the manner described above. In experiments 76, 79 and 80 a strength of current six times that necessary to cause ocular changes characteristic of sympathetic stimulation was used; in all of the other experiments to be found in table 3 the current was of the same strength as that used in the experiments recorded in table 2. From the standpoint of the iodine concentration in whole gland the results given in tables 2 and 3 are very similar. Stimulation apparently has no effect on the concentration of iodine in the stimulated lobe.

By stimulating the intact vago-sympathetic nerve Rahe et al. (2) declare that they were able to produce the most marked diminution in the iodine content of a given lobe of the thyroid gland. In the three experiments of table 4 of my series, the intact vago-sympathetic nerve was

TABLE 3

The concentration of iodine in the lobes of the thyroid gland of the dog after the stimulation of the isolated vago-sympathetic nerve on one side for a period of approximately three hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	WEIGHT OF DRIED WHOLE GLAND	IODINE IN DRIED WHOLE GLAND	APPARENT GAIN (+) OR LOSS (−) IN CONCENTRATION OF IODINE IN STIMULATED LOBE
			gram	*per cent*	*per cent*
72	Right	2 hrs. 50 min.	L 1.2883	0.186	+0.003
			R 1.4258	0.189	
74	Right	3 hrs. 10 min.	L 2.0197	0.381	−0.008
			R 1.5013	0.373	
75*	Left	3 hrs. 10 min.	L 1.4975	0.334	−0.026
			R 1.8796	0.360	
76	Right	3 hrs. 10 min.	L 0.4653	0.770	+0.051
			R 0.3509	0.821	
77	Right	3 hrs. 30 min.	L 0.7055	0.471	+0.037
			R 0.7914	0.508	
79	Right	3 hrs. 15 min.	L 0.2421	0.474	+0.024
			R 0.1995	0.498	
80	Left	4 hrs. 0 min.	L 0.6342	0.465	−0.012
			R 0.4859	0.477	
82	Left	3 hrs. 10 min.	L 0.8483	0.203	−0.003
			R 0.7516	0.206	
83	Left	3 hrs. 15 min.	L 1.2435	0.105	+0.002
			R 0.9163	0.103	
84	Left	3 hrs. 10 min.	L 0.7582	0.129	−0.016
			R 0.6022	0.145	

* Entire right lobe not analyzed.

stimulated in the neck at about the level of the fifteenth tracheal ring. The current was of the same strength as that employed in the experiments of table 2; each time the current was made there ensued a respiratory arrest and the ocular changes typical of sympathetic stimulation. Again there was no consistent change in the concentration of iodine in the stimulated lobe.

TABLE 4

The concentration of iodine in the lobes of the thyroid gland of the dog after the stimulation of the intact vago-sympathetic nerve on one side for a period of approximately three hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	WEIGHT OF DRIED WHOLE GLAND	IODINE IN DRIED WHOLE GLAND	APPARENT GAIN (+) OR LOSS (−) IN CONCENTRATION OF IODINE IN STIMULATED LOBE
			gram	*per cent*	*per cent*
78	Left	3 hrs. 40 min.	L 0.4628 R 0.3706	0.528 0.501	+0.027
86	Right	3 hrs. 20 min.	L 0.7039 R 0.6919	0.138 0.125	−0.013
88	Left	3 hrs. 15 min.	L 0.9752 R 1.2385	0.127 0.123	+0.004

Effect of vasomotor activity on the concentration of iodine in the thyroid gland. It will be recalled that Watts (3) concluded from his experiments that vascular changes will account for the diminution in the concentration of iodine which he brought about by stimulation of the cervical sympathetic nerve. The experiments of table 5 were undertaken to find out whether or not a slightly different type of stimulus sent into the nerve at an interval more nearly like that employed by Watts had an effect comparable to that found in the experiments previously performed. It was also thought desirable to determine whether or not the characteristic vascular changes were present throughout the experiments.

The technique except for certain features of the stimulation was the same as that used in all of the preceding experiments. Usually the stimulated vago-sympathetic nerve was ligated and cut at about the level of the eighteenth to twentieth tracheal ring; near the ganglion nodosum only the vagus nerve was cut. In all of the experiments the sympathetic chain was intact above the eighteenth tracheal ring. The

TABLE 5

The concentration of iodine in the lobes of the thryoid gland of the dog after the stimulation of the vago-sympathetic nerve, the sympathetic portion of which was left intact above the point of stimulation

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	PRESENCE (+) OR ABSENCE (—) OF VASOMOTOR ACTIVITY AT END OF EXPERIMENT		WEIGHT OF DRIED WHOLE GLAND	IODINE IN DRIED WHOLE GLAND	APPARENT GAIN (+) OR LOSS (—) IN CONCENTRATION OF IODINE IN STIMULATED LOBE
					grams	*per cent*	*per cent*
101	Right	3 hrs. 10 min.	—	L	1.8213	0.177	−0.052
				R	1.5890	0.125	
102	Left	3 hrs. 0 min.	+	L	0.1397	0.141	+0.021
				R	0.1727	0.120	
103	Right	3 hrs. 0 min.	+	L	0.6335	0.277	−0.003
				R	0.5749	0.274	
104	Right	3 hrs. 0 min.	—	L	0.1891	0.190	−0.026
				R	0.1844	0.164	
105	Right	3 hrs. 25 min.	+	L	1.4379	0.167	−0.011
				R	0.9005	0.156	
106	Left	3 hrs. 25 min.	+	L	0.6161	0.043	+0.002
				R	0.6624	0.041	
107	Right	3 hrs. 0 min.	—	L	3.1065	0.103	+0.006
				R	2.9642	0.109	
108	Left	3 hrs. 35 min.	+	L	0.3462	0.021	−0.006
				R	0.2754	0.027	
109	Right	3 hrs. 25 min.	+	L	1.0407	0.014	+0.002
				R	1.0116	0.016	
111	Left	3 hrs. 50 min.	+	L	0.2875	0.076	+0.001
				R	0.2638	0.075	
113	Right	3 hrs. 30 min.	—	L	1.5649	0.034	−0.001
				R	2.6585	0.033	
114	Right	3 hrs. 30 min.	+	L	0.3210	0.143	−0.011
				R	0.3690	0.132	
115	Left	3 hrs. 45 min.	+	L	1.0381	0.088	−0.002
				R	0.8196	0.090	
116	Left	3 hrs. 30 min.	—	L	0.6650	0.016	−0.001
				R	0.6753	0.017	
117	Left	4 hrs. 20 min.	+	L	1.1050	0.140	+0.006
				R	1.1701	0.134	
118	Right	5 hrs. 0 min.	—	L	0.6568	0.456	−0.018
				R	0.5036	0.438	
119	Right	3 hrs. 10 min.		L	0.6866	0.168	+0.005
				R	0.5695	0.173	

vago-sympathetic nerve was stimulated just above the point at which it was cut near the eighteenth tracheal ring. It was easily possible to vary the strength of the electrical stimulus so that a current of such a strength was employed as just to bring about the ocular changes characteristically associated with stimulation of the cervical sympathetic nerve. Mendenhall (15) has emphasized the markedly toxic effects of ether on the sympathetic nervous system. In a preparation like that last described the sensitivity of the cervical sympathetic nerve to the depressant action of ether could easily be demonstrated. A tetanizing current of rather low frequency from a Stoelting inductorium was made for 5.5 seconds at intervals of 11.8 seconds throughout the period of stimulation. At the end of most of these experiments a vein of the stimulated lobe, in most cases without the ligation of its companion veins, was cannulated and the effect on blood flow of stimulation of the gland under the *same* conditions as those employed in the experiment was observed. In ten out of sixteen experiments no difficulty was encountered in demonstrating a vasoconstriction in the gland on stimulating the vago-sympathetic nerve with an electric current of the same strength and delivered at the same rate as that used in the previous stimulation period. The threshold of excitability for the vasoconstrictor nerves of the thyroid gland appears to be considerably lower than that for the submaxillary gland as reported by Gruber (16). The relatively low threshold of excitability of the vasoconstrictor nerves of the thyroid to epinephrin stimulation has been observed by Gunning (17).

From table 5 it may be seen that stimulation of the vago-sympathetic nerve with the sympathetic trunk intact above the eighteenth tracheal ring has no appreciable effect on the concentration of iodine in the stimulated lobe. In a number of experiments it was unequivocally demonstrated that vasoconstrictor fibers to the gland were being stimulated; yet such stimulation did not alter detectably the iodine content of the lobe subjected to stimulation.

Effect of stimulation of the vago-sympathetic nerve on the concentration of water in the thyroid gland. In table 6 may be found data relative to the effect of stimulation of the vago-sympathetic nerve on the concentration of water in the thyroid gland. I was unable to find that stimulation had any effect on the concentration of water in the stimulated lobe even in experiments in which vasomotor changes were definitely proved to result from stimulation.

TABLE 6

The concentration of water in the lobes of the thyroid gland of the dog after the stimulation of the vago-sympathetic nerve for a period of from three to four hours

ANIMAL NUMBER	LOBE STIMULATED	LENGTH OF PERIOD OF STIMULATION	CONCENTRATION OF WATER	APPARENT GAIN (+) OR LOSS (—) IN CONCENTRATION OF WATER IN STIMULATED LOBE
			per cent	*per cent*
77	Right	3 hrs. 30 min.	L 69.25 R 69.64	+0.39
79	Right	3 hrs. 15 min.	L 73.40 R 74.26	+0.86
82	Left	3 hrs. 10 min.	L 74.06 R 74.66	−0.60
83	Left	3 hrs. 15 min.	L 77.57 R 78.02	−0.45
84	Left	3 hrs. 10 min.	L 74.38 R 74.24	+0.14
88	Left	3 hrs. 15 min.	L 73.65 R 72.81	+0.84
108*	Left	3 hrs. 35 min.	L 80.14 R 80.71	−0.57
109*	Right	3 hrs. 25 min.	L 78.54 R 80.64	+2.10
110*	Right	3 hrs. 45 min.	L 82.69 R 82.13	−0.56
111*	Left	3 hrs. 50 min.	L 78.03 R 77.33	+0.70
113	Right	3 hrs. 30 min.	L 75.98 R 73.73	−2.25
115*	Left	3 hrs. 45 min.	L 75.76 R 75.63	+0.13
116	Left	3 hrs. 30 min.	L 79.92 R 78.34	+1.58
117*	Left	4 hrs. 20 min.	L 76.53 R 75.65	+0.88
119	Right	3 hrs. 10 min.	L 75.90 R 76.81	+0.91

* Vasomotor effect of the stimulation demonstrated at the end of the experiment.

DISCUSSION

If we consider the data described above we find that following the stimulation of a given lobe of the thyroid gland there was no consistent change in the concentration of iodine in that lobe compared with the

TABLE 7

A comparison of the effect of the different methods of stimulation of the vago-sympathetic nerve on the concentration of iodine in the thyroid gland of the dog

SERIES	NON-SIGNIFICANT	APPARENT LOSS IN CONCENTRATION OF IODINE			APPARENT GAIN IN CONCENTRATION OF IODINE			REMARKS
		Number	Maximum	Average	Number	Maximum	Average	
			per cent	*per cent*		*per cent*	*per cent*	
I	2	7	0.060	0.035	5	0.046	0.028	Stimulated vago-sympathetic nerve cut above hyoid bone and low in the neck. Non-stimulated vago-sympathetic nerve cut similarly in most cases
II	4	3	0.026	0.018	3	0.051	0.037	Only stimulated vago-sympathetic nerve cut as in series I
III	1	1	0.013	0.013	1	0.027	0.027	Intact vago-sympathetic nerve stimulated at about level of fifteenth tracheal ring
IV	11	5	0.052	0.024	1	0.021	0.021	Vago-sympathetic nerve stimulated at about level of eighteenth tracheal ring. Sympathetic nerve entirely intact above point of stimulation
V	7	2	0.011	0.011	1	0.021	0.021	Animals of series IV in which vasomotor effect of stimulation could be shown plainly at end of experiment

nonstimulated lobe. Following stimulation there was an apparent diminution in the concentration of iodine in 24 or 54.5 per cent of the stimulated glands, and an apparent gain in 20 or 45.5 per cent of glands stimulated similarly. Watts (3) reported that the average difference

in the percentage of iodine in the lobes of dried thyroid gland of the dog is 0.015 per cent in this vicinity. If we consider as non-significant all differences in the percentage of iodine in dried thyroid of 0.010 per cent or less we see that stimulation, while causing an apparent diminution in the concentration of iodine in 16 or 36.4 per cent of the glands, and an apparent increase in the concentration of iodine in 10 or 22.7 per cent of the glands, had no effect on the iodine concentration of 18 or 40.9 per cent of the stimulated glands. The greater part of the experimental data presented may be briefly summarized in table 7.

The differences which I found above appear to be due to normal variations in the concentration of iodine in the two lobes of the dog's thyroid gland. The average percentage difference in the concentration of iodine in the two lobes of the dog's thyroid gland depends on a number of factors such as the type of gland, the time of year and the feeding of iodine. Hence I am forced to conclude that if stimulation of the vago-sympathetic nerve in the dog has any effect on the concentration of iodine in the thyroid gland, that effect is considerably less than the normal variation in the iodine content of the stimulated and control lobes. The presence of normal variations relatively so much greater than the variations which may follow stimulation renders valueless the application of more refined methods of iodine determination in the study of the effect of stimulation of the cervical sympathetic nerve on the concentration of iodine in the thyroid gland.

SUMMARY

1. Periodic stimulation of the isolated vago-sympathetic nerve by an induced current of a moderate to strong intensity over a period of from three to three and a half hours does not appreciably alter the distribution ratio of iodine between cells and colloid.

2. The findings of Rahe, Rogers, Fawcett and Beebe (2) and of Watts (3) that stimulation of the cervical sympathetic nerve for a comparable period of time reduces the concentration of iodine in the stimulated lobe were not confirmed. Watts' assertion that such stimulation reduces the water content of the stimulated lobe was not confirmed.

3. Conclusions as to the direct secretory control which the cervical sympathetic nerves exercise on the thyroid gland are based in no small measure on the alleged effect of stimulation of the cervical sympathetic nerve on the iodine content of the gland. Conclusions having such a basis apparently are untenable.

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EXTENSIBILITY OF MUSCLE: THE EFFECT OF STRETCHING UPON THE DEVELOPMENT OF FATIGUE IN A MUSCLE

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The extensibility of muscle was first systematically investigated by Weber (1) in 1846. It was he who found that a muscle stretched by the addition of equal increments of weight does not follow Hooke's law. Its curve of extensibility is not a straight line but a hyperbola. Weber's findings have been confirmed beyond question. There are, however, some other organic substances, non-living, which do not follow Hooke's law when extended by weights (2). Therefore, is the muscle which is stretched by a weight purely passive, or, does it put forth an active effort to support the weight; that is, does the stretching of a living muscle involve an increase in its metabolism, an energy transformation, the doing of work?

Guerrini (3) considers the specific form of the curve of extensibility of muscle as affording evidence of a vital response in the muscle. He speaks of the later more gradual part of the curve as dependent upon the biological factor, arguing from the lessening or total disappearance of this part of the curve, the straightening out of the curve in other words, when the vitality of the muscle is lessened or destroyed. He cites particularly poisoning of the muscle by various means and fatty degeneration of the muscle as affecting the curve in this manner. Many observers are agreed that the extensibility of muscle is increased, the curve more nearly approaches a straight line, during contraction and with the development of fatigue.

Brodie (4) found that muscles in situ with intact nerve and blood supply were more resistant to extending forces, the same weight caused less lengthening, than isolated muscles or muscles in situ whose nerve supply had been cut.

Rood (5) in 1861 claimed to have demonstrated that a sudden stretching may serve as a stimulus and cause the muscle to contract. Fick (6), on the other hand, emphatically denied such a possibility. We have ourselves observed the phenomenon to take place in some of our experiments. If simply an incision is made over the gastrocnemius tendon in a decerebrated frog and the tendon freed and cut without dissection of the muscle, or interference with its nerve and blood supply, the application of a small extending force, 10 or 20 grams, is not infrequently followed immediately by a contraction of the muscle. We have seen the tone of the muscle so increased that its actual length was less than before the weight was applied.

Montgomery (7) appears to have been the first to conceive the possibility that the stretching of a muscle may be accompanied by chemical as well as physical changes within the muscle. He did not, however, venture to reveal the nature of these chemical changes.

Meyerstein and Thiry (8), Schmulewitsch (9), Westermann (10), Blix (11), Danilewsky (12) and Roy (13) all found that there is a rise of temperature in a muscle when it is stretched. Danilewsky, it is true, attempted to explain the temperature change as due to internal friction and Westermann and Roy observed qualitatively similar elevation of temperature in dead muscle and other tissues removed at autopsy; but the other authors quoted believed that there was an actual increased production of heat coincident with the extension.

Hill (14) also has recently recorded the production of heat when a muscle is extended by a weight. He gives to this heat production a twofold explanation: Based upon a mathematical formula the temperature should rise because the coefficient of thermal expansion for muscle is negative; there is a wasting of work with liberation of heat in overcoming viscous forces when the muscle is stretched at a finite rate.

Du Bois Reymond (15) and v. Koränyi and Vas (16) reported a change in the electrical condition of muscle, a negative variation, when it is "regularly" stretched.

Wundt (17) and Nasse (18) found in their experiments that the muscle which has been stretched fatigues more quickly upon subsequent stimulation than one which has not been stretched. On the other hand, the possibility of a fatiguing effect of mere stretching has been denied by Kronecker (19), Harless (20) and Leber (21). It should be pointed out that the last two authors employed small weights and allowed them to act for short periods only.

Taking up the question at this point we have carried out a series of twenty-five experiments with a view to determining the possible extent to which the stretching of a muscle may influence the development of fatigue.

Both gastrocnemius muscles were removed from a pithed frog as speedily as possible with especial care to avoid traction on either muscle. Each was fastened in the femur clamp of a separate moist chamber of the Harvard type and fine wires were run from the base of the moist chamber to the muscle for its direct stimulation. A pin-hook with attached thread was passed through each tendo Achillis. One was left free; the other was fastened to a muscle lever and made to support a weight of 100 grams for varying lengths of time. Subsequently each muscle was stimulated with an induced current to the point of complete exhaustion. In two instances, experiments 4 and 21, a tetanizing current was employed; in all of the other experiments the stimuli were single break shocks. In the first seven experiments the stretched and unstretched muscles were connected in series in one secondary circuit of an induction coil so that both muscles were stimulated simultaneously. The strength of the stimulus was that which called forth maximal response in the unstretched muscle. The stimuli were sent in at the rate of one per second. The direction of the current in the secondary circuit was varied so that in some experiments the stretched muscle and in others the unstretched muscle was first traversed by the current.

In the remainder of the experiments the two muscles were stimulated separately. It made no difference in the results whether the unstretched muscle was stimulated first or allowed to hang in the moist chamber until after the intervals of stretching and stimulation of the other muscle. For these experiments the strength of the stimulus was approximately the maximal break shock. Exact determination was not made to avoid delay, our judgment being based upon previous experience. The stimulus was of course the same for both muscles of a pair. The minimal stimulus for each muscle was determined in some of the experiments.

The stimulation of the stretched muscle was always carried out immediately upon the removal of the weight. The muscle was always left attached to the muscle lever and sometimes additional 20 or 30 grams were suspended from it. The unstretched muscle was connected to the muscle lever just prior to its stimulation and a load of 20 or 30 grams added if necessary to make the conditions uniform. Usually the writing point of the muscle lever was brought against the smoked

surface of the kymograph and the contractions recorded. The time required to effect complete exhaustion of each muscle was determined with a stop-watch. The results of these experiments are set forth in table 1.

TABLE 1

EXPERIMENT NUMBER	STRETCHING FORCE	DURATION OF STRETCHING	MINIMAL STIMULAS DISTANCE OF SECONDARY COIL FROM PRIMARY		LOAD	FATIGUE TIME	
			Stretched muscle	Unstretched muscle		Stretched muscle	Unstretched muscle
	grams	*minutes*	*cm.*	*cm.*	*grams*	*minutes*	*minutes*
1	100	30			20	17.76	20.15
2	100	30			20	14.08	19.50
3	100	30			30	4.86	6.06
4	100	30			30	3.25	5.41
5	100	30			None	10.40	10.40
6	100	30			None	16.86	17.25
7	100	30			None	14.96	15.75
8	100	30			20	18.50	20.50
9	100	30			30	15.36	15.36
10	100	30			20	11.51	16.70
11	100	60			20	12.63	17.36
12	100	60	21.6	15.7	30	11.76	10.20
13	100	90	18.5	22.8	None	27.08	38.83
14	100	45	20.5	19.5	None	38.75	46.88
15	100	45	21.9	22.4	20	22.75	25.05
16	100	120	25.1	26.3	20	13.40	10.55
17	100	120	18.5	21.7	30	13.30	11.75
18	100	120	19.3	21.2	None	38.66	46.16
19	100	165	23.6	19.6	None	25.15	19.50
20	100	195	26.1	27.1	20	10.08	13.66
21	100	60	23.3	19.8	None	10.08	10.76
22	100	180	19.5	17.3	20	16.41	17.93
23	100	150	21.1	24.5	None	16.08	19.11
24	100	120	21.4	19.7	None	20.16	37.08
25	100	140	28.0	20.1	20	9.63	9.35
Averages.....			22.0	21.2		16.53	19.25

We find that the average time for complete exhaustion of the muscle which had been extended by a weight of 100 grams for periods of 30 minutes to 3 hours was 16.53 minutes, and of the unstretched muscle which had been kept in moist air for similar periods of time was 19.25 minutes; that is, the former was completely fatigued in 14.02 per cent less time than the latter. We find, by comparison of the experiments

in which the interval of stretching was only 30 minutes with the others in which the extending force was allowed to act longer, that the effect was to a slight degree increased by the longer periods of stretching. In the former group the stretched muscle was completely fatigued in 13.46 per cent less time than the unstretched muscle and in the latter group in 14.41 per cent less time. When each muscle was required to lift a load of 20 or 30 grams at each contraction the difference in the fatigue time was 11.14 per cent; when the muscles were not loaded during the time of stimulation the stretched muscle was exhausted in 16.66 per cent less time than the other.

These results would seem to be sufficiently marked to justify the conclusion that a previous stretching hastens the onset of fatigue in a muscle. This is not due simply to an alteration in the irritability of the muscle since the average minimal stimulus is slightly weaker for the stretched than for the unstretched muscle. Obviously there is no opportunity for the isolated muscle to replenish its store of energy-yielding material and the using up of some of this by the muscle in combating the stretching force would hasten the development of fatigue since fatigue is not only a matter of accumulation of waste products but also an exhaustion of energy-yielding material.

CONCLUSIONS

1. The gastrocnemius muscle of the frog which has been stretched by a weight of 100 grams for periods of 30 minutes to 3 hours is completely exhausted by subsequent stimulation in less time than an unstretched muscle kept in moist air for similar periods of time.

2. This difference is increased slightly by increasing the duration of the stretching.

3. The stretching does not uniformly affect the irritability of the muscle.

4. It is suggested that the muscle uses up some of its available store of energy-yielding material in combating the stretching force and that the development of fatigue is hastened for this reason.

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EXTENSIBILITY OF MUSCLE: THE PRODUCTION OF CARBON DIOXIDE BY A MUSCLE WHEN IT IS MADE TO SUPPORT A WEIGHT

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In a previous paper (1) we showed that the gastrocnemius of the frog, when stretched by a weight of 100 grams for ½ to 3 hours, can be completely fatigued in 85.98 per cent of the time required in the case of an unstretched muscle, when both are stimulated by the same strength induced current. We suggested that this effect of stretching might result because the muscle uses up some of its available reserve of energy-yielding material in combating the extending force, and that the muscle, to a certain extent at least, puts forth an active effort to support the weight. One would expect such an effort to involve the consumption of oxygen and production of carbon dioxide. The following experiment is indirect evidence of oxygen consumption under these circumstances.

We arranged two moist chambers with air-tight covers and provided circulation of oxygen through one and of nitrogen through the other. The flow of gases was regulated so as to be uniform in rate and pressure in the two chambers. Both gastrocnemii of a pithed frog were prepared and fastened in the femur clamps, one in the oxygen chamber, the other in the nitrogen chamber. After the flow of gases had been established and time allowed for air displacement in the apparatus, both muscles were loaded with equal weights and the amount of stretching of each in equal periods of time recorded. In six such experiments the actual lengthening of the muscle in oxygen averaged 4.05 mm. and of the muscle in nitrogen 5.08 mm.; the latter had stretched 25.43 per cent more than the former. Deprived of oxygen, therefore, the muscle is less resistant to the stretching force. Similarly Brodie (2) found that muscles in situ with intact nerve and blood supply were more resistant to stretching than isolated muscles. It is also true that in our experiments and in those of Brodie where the extension was greatest the recovery on removal of the weight was least perfect.

Gotschlich (3) in 1894 investigated the possible production of lactic acid by a muscle when it is stretched. He allowed weights of 100 to 1000 grams to extend one gastrocnemius of the frog (the other muscle was used as a control) for 1 hour. He then made muscle extracts in physiological saline solution and examined these extracts for lactic acid using the sodium salt of alizarin as indicator. He found uniformly no evidence of lactic acid in the unstretched muscle. He always found lactic acid in the muscle which had been stretched, the amount present being roughly proportional to the stretching force. It was also increased when the extension was effected at a higher temperature, 25°C. as compared with 11°C. When the load was 1000 grams, extending the muscle for 1 hour, he reports the detection of 1.69 mgm. of lactic acid per gram of muscle. Gotschlich says, "With maximal load and a temperature of 25°C. the quantity of acid was almost as great as that produced by a series of two hundred maximal contractions." He observed also that the acidity was still further increased by rhythmical loading and unloading over that resulting from constant stretching.

Garrey (4) notes that muscles which have been stretched increase in weight more rapidly than unstretched muscles when immersed in physiological saline solution. The difference is particularly marked when large weights are allowed to act for considerable periods of time. Fletcher (5) has shown that fatigued muscles, similarly immersed, increase in weight by the taking up of water more rapidly than resting muscles. He explains this as due to increased osmotic pressure within the muscle on account of the breaking up of substances during contraction. Accordingly Garrey interprets his findings as indicating an increased osmotic pressure in the stretched muscle from a like cause.

We have determined the amount of carbon dioxide produced in stretched and unstretched muscles by employing the principles of the apparatus devised by Tashiro (6), (7). The chief modification necessary for our purpose was an increase in the capacity of the apparatus. The services of a glass-blower were not available so that we have made use of such pieces of ready made glassware as were available in the laboratory. The set-up is shown in figure 1 and consisted in brief of the following parts:

A twelve liter bottle, *A*, containing 20 per cent solution of sodium hydroxide is connected through *1* with another twelve liter bottle, *B*, which holds at the beginning a small amount of 20 per cent sodium hydroxide solution. The air in *B* is vigorously shaken with the hydroxide solution, completely removing the carbon dioxide. By siphoning over more hydroxide solution from *A* this carbon

dioxide free air is driven out of *B* as required into the container, *D*, a 2,000 cc. flask. This flask is in siphon relation with the open wide-mouthed bottle, *C*, containing 5 per cent sodium hydroxide solution. Weak hydroxide solution is used so that the chamber will not be too dry and at the same time contamination of the carbon dioxide free air in *D* will be prevented. By means of *C* the air in *D* and in fact throughout the remainder of the apparatus is kept at atmospheric pressure. The chambers, *G* and *G'*, cylindrical separatory funnels, were provided with carefully fitted stoppers from the under surface of which the muscles were suspended. These stoppers were so arranged that they might be mercury sealed. Into each chamber was run a tube with upturned end, *X* and *X'*, to form a cup for the drop of barium hydrate solution. Connected with *G* and *G'* was a mercury reservoir, *H*, for regulating their capacity. Also connected with *G* and *G'* respectively were graduated burettes, *I* and *I'*, by which measured small quantities of

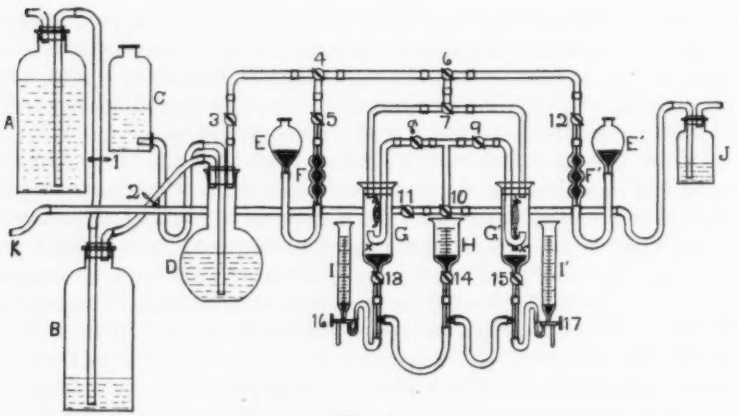

Fig. 1

mercury could be withdrawn as required in the quantitative determination of the carbon dioxide produced. The blood gas bulbs, *F* and *F'*, with the mercury pressure funnels, *E* and *E'*, were used for the withdrawal of air from *G* and *G'* and its subsequent replacement in the quantitative analysis. A bottle, *J*, containing saturated solution of barium hydrate was connected with the tubing terminating at *X* and *X'*, and an efficient electric vacuum pump was connected on at *K*. Heavy walled capillary glass tubing and stopcocks were used throughout, except the terminal part of the barium hydrate tubes, *X* and *X'*, where 3 mm. bore tubing was used. All rubber connections were made as short as possible and all joints were rendered air-tight by repeated coating with shellac.

The first step in the experimental procedure consisted in the testing of the apparatus for its air-tightness and the absolute freedom from carbon dioxide of the air supplied from *B*.

With the apparatus clean and dry stopcocks *4*, *6* and *7* were turned so as to make connection between *D* and *G*, *G'* only. Stopcock *3* was closed, stopcocks *8*, *9* and *11* were opened and stopcock *10* was turned to connect the upright tube with *11*, excluding the tube from *J*. The air was now exhausted from the chambers *G* and *G'* by the pump at *K*. Stopcock *11* was closed and stopcock *3* opened permitting carbon dioxide free air to enter from *D* to fill the vacuum created in *G* and *G'*. The pressure was made equal to atmospheric pressure by adjusting the level of *C*. This process of evacuating *G* and *G'* and refilling with CO_2-free air was always repeated at least four times. The efficiency of the apparatus depends very largely upon the thoroughness of this washing-out process. When *G* and *G'* finally contained pure CO_2-free air barium hydrate solution was let in from *J* by turning stopcock *10* in the proper direction (*11* was closed) and very gradually opening stopcocks *8* and *9* in turn until a hemispherical drop of clear barium hydrate solution stood on the upturned tubes at *X* and *X'*. Stopcocks *8* and *9* were closed, the pressure quickly adjusted to atmospheric pressure at *C* and stopcock *3* was closed. If the air is absolutely free of carbon dioxide the drops of barium hydroxide solution remain clear indefinitely. On the other hand, if carbon dioxide is present, even as little as 1×10^{-7} grams will cause precipitation within 10 minutes of particles of barium carbonate in the drop of barium hydrate and larger amounts will cause increase in the size and number of the particles (7). The precipitate is detected by observation of the drop through a magnifying lens.

In our apparatus the drop of barium hydrate obtained in the manner described remained clear over night in repeated tests, proving beyond question that the air in *B* was absolutely carbon dioxide free, that the washing out process was thorough and complete, and that there were no leaks.

Oxidation processes which would lead to the production of carbon dioxide have been shown by various investigators,—Herman (8), Hill (9), Gyllenswärd (10)—to occur in the surviving isolated muscles of the frog. To prove liberation of carbon dioxide in a muscle under the influence of a stretching force it is necessary to demonstrate, therefore, that such a muscle produces carbon dioxide more rapidly or in greater amount than a muscle of the same size under the same conditions of temperature, atmospheric pressure and surrounding medium. It was for a comparison of this sort that the two chambers, *G* and *G'*, were provided. It was further necessary for an exact comparison to have the capacity of the two chambers identical. For this purpose both chambers were stoppered in the usual manner after placing in one the weight to be subsequently used to stretch the muscle (an ordinary 50-gram weight with a wire ring attached). Then the chambers were completely filled with mercury from *H*, exactly 150 cc. of mercury were withdrawn from each and the stopcocks *13*, *14* and *15* closed, leaving a safety

layer of mercury in the bottom of each chamber. The gas capacity of G was, therefore, exactly equal to the gas capacity of G' before the introduction of the muscles.

Both gastrocnemii of a pithed frog were prepared in the usual manner, care being exercised to cut the tendon, the tibia and the femur at the same points in both preparations. Except in a very few of the early experiments all the muscular tissue about the knee joint except the gastrocnemius was carefully dissected away. An S-shaped pin-hook was passed through the knee joint and another through the tendon of each preparation. Each muscle was hung from a hook on the under surface of the stopper of its chamber; the 50-gram weight was hung from one of the muscles; the stoppers were put into position and sealed. The air was exhausted and G and G' refilled with CO_2-free air by the washing out process described in connection with the testing of the apparatus. The drops of barium hydrate were got into position; pressure in various parts of the apparatus was equalized by adjustment at C; and very careful watch was kept for the first appearance of precipitate in the barium hydrate. Twenty-one such observations were made.

Averaging the results of these experiments we find that 4.07 minutes elapsed between the placing of the drop of barium hydrate in position in the chamber containing the stretched muscle and the first appearance of precipitate, whereas the interval was 17.47 minutes in the case of the unstretched muscle. In the individual experiments the results varied between 2 and 10 minutes for the stretched muscle and between 10 and 30 minutes for the unstretched muscle. In every instance the drop of barium hydrate in relation with the stretched muscle showed precipitate much sooner than that in relation with the unstretched muscle.

As a further check the use of the chambers was alternated from time to time with proper volume correction. Since we used companion muscles from the same frog it seems justifiable to disregard any slight differences there might have been in the two muscles under identical tension conditions. In nearly all cases, as pointed out, all muscular tissue other than the gastrocnemius was dissected away avoiding any variation from this source. Theoretically the surface area of the muscle might be increased by stretching and with increased surface area there might be greater diffusion outward of carbon dioxide without necessarily increased production. But actual measurement showed that there was sometimes no change at all and at other times a slight, usually insignificant, increase in surface area.

We have made but one quantitative determination of the carbon dioxide produced by the stretched and unstretched muscles, again following the method described by Tashiro (7).

With stopcock *3* closed and all the other stopcocks turned to make connection between F, F' and the tube to the pump, E and E' were raised until a bit of mercury was forced from each direction into the capillary tube between stopcocks *4* and *6*. Stopcocks *5* and *12* were now closed and stopcocks *4* and *6* turned cutting off the connection to F and F' respectively. E and E' were kept elevated so that the mercury in them was at least up to the level of the upper horizontal tubing. The mercury in the tubing between stopcocks *4* and *6* was displaced into G, G' by greatly elevating C and was withdrawn into I, I' so as to restore the correct volume in the muscle chambers.

The two gastrocnemius muscles were prepared as in the other experiments, placed in position in the chambers G and G' with the 50-gram weight suspended from the muscle in G' and the washing out process gone through with in the way already described. At the expiration of 60 minutes stopcocks *4*, *5*, *6* and *7* were turned to make connection between G and F only. Then, stopcocks *13* and *14* having been opened, by raising H mercury was run into G and the air displaced into F. It was of course necessary to lower E at the same time sufficiently to maintain atmospheric pressure. After the expulsion of all of the air in G stopcock *5* was closed, mercury was again withdrawn from G to restore its volume to 150 cc. and stopcock *13* was closed. In like manner the air in G' was transferred into F'. The muscles were removed, the stoppers replaced and sealed, the washing out process repeated and drops of barium hydrate solution got into position at X and X' when the chambers finally contained CO_2-free air. The pressure was made equal to atmospheric pressure by adjustment at C; stopcocks *3*, *8* and *9* were closed. Stopcocks *4*, *5*, *6* and *7* were turned to make connection between F and G (the chamber which had contained the unstretched muscle). By opening stopcock *13* and turning stopcock *16* at the bottom of the graduated cylinder I, measured volumes of mercury were withdrawn from G permitting air to enter from F with readjustment of pressure by raising E as necessary. In this manner 5 cc. of air were transferred from F into G. Ten minutes later, the drop of barium hydrate being still clear, another 5 cc. of air were transferred into G. We continued to transfer 5 cc. of air at a time at 10-minute intervals until the thirteenth instalment (a total of 65 cc.) was introduced. Three minutes later precipitate began to form at X. By the same method air was transferred from F' to G' (the chamber which had contained the stretched muscle) in 5 cc. increments at 10-minute intervals. Three and a half minutes after the introduction of the third 5 cc. increment, precipitate first appeared at X'. The quantity of air containing the minimum amount of carbon dioxide which will precipitate barium carbonate when diluted by 150 cc. of CO_2-free air was, therefore, approximately 65 cc. when using air in which the unstretched muscle had been suspended and 15 cc. when using air in which the stretched muscle had been suspended. To arrive more closely at the minimum quantity of air required to produce a precipitate in the two cases the capacity of each of the chambers was restored to 150 cc. by introduction of mercury from H, the washing-out process

was repeated and drops of barium hydrate again got into position at X and X'. Sixty cubic centimeters of air were run in at once from F to G by removing mercury into I. Ten minutes later the drop was still clear. Then 1 cc. of air at a time was introduced at ten minute intervals. Six minutes after the introduction of the second 1 cc. increment precipitate began to form at X. Ten cubic centimeters of air were transferred from F' to G' and 1 cc. at a time at 10-minute intervals until precipitate began to form at X', 2 minutes after the introduction of the fourth cubic centimeter. Therefore, 62 cc. of air from the chamber which had held the unstretched muscle and 14 cc. of air from the chamber which had held the stretched muscle each contained the minimum quantity of carbon dioxide necessary to cause a precipitate in the drop of barium hydrate, when added to 150 cc. of CO_2-free air.

According to Tashiro (6) the minimum quantity of carbon dioxide which will cause a precipitate to form within 10 minutes in the drop of barium hydrate is 1×10^{-7} grams when the total volume of the chamber in which the analysis is made is 15 to 25 cc. In our experiments, where the total volume of the analysis chamber was 150 cc., the minimum carbon dioxide value will be at least as great, possibly greater. Using Tashiro's figures, however, as equivalent to the 62 cc. in the one case and 14 cc. in the other, we calculate the total carbon dioxide produced by the unstretched muscle in 60 minutes as $\frac{150}{62} \times 10^{-7}$ or 2.4×10^{-7} grams, and by the stretched muscle in the same period as $\frac{150}{14} \times 10^{-7}$ or 10.7×10^{-7} grams; the latter had produced 4.45 times as much as the former.

CONCLUSIONS

1. The isolated gastrocnemius muscle of the frog when extended by a weight of 50 grams produces carbon dioxide at a faster rate than an unstretched muscle otherwise under the same conditions.

2. In a 60-minute period the stretched muscle produces about four and a half times as much carbon dioxide as the unstretched muscle.

3. The increased carbon dioxide production is direct evidence that the stretched muscle puts forth an active effort to support the weight.

The experiments herein described were carried out chiefly in the Department of Physiology of McGill University and our thanks are due Prof. John Tait for the courtesies extended.

We wish to acknowledge our indebtedness to Mr. Charles A. Eddy for making the diagram which accompanies this report.

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STUDIES OF THE THYROID APPARATUS

I. The Stability of the Nervous System as a Factor in the Resistance of the Albino Rat to the Loss of the Parathyroid Secretion

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This paper is a report and an interpretation of the differences of resistance of two groups of albino rats to the loss, by operation, of the parathyroid secretion. No general review of previous studies of the parathyroids is given and reference is made only to the landmarks and investigations pertinent to the present subject, since that mass of material has been thoroughly covered by other workers (1).

Gruber (2), in 1845, was probably the first to call attention in the literature to those little groups of cells anatomically associated with the thyroid gland which we now know as the parathyroids from the name given to them by Sandström (3) in 1880, and around the function of which in connection with thyroid function there raged that vivid controversy between Gley (4) and Moussu (5). Later work has established definitely that the parathyroids do possess a distinct function independent of that of the thyroid. Whether or not the parathyroids possess functions that are complementary, supplementary or antagonistic to any thyroid functions is not germane to the present discussion.

The discovery of the parathyroid glands in the albino rat can be credited to Cristiani (6), who reported that this animal possessed two of these glands, one associated with each of the two lobes of the thyroid; thus distinguishing it from many other mammalia, most of which have four. Although Erdheim (7) claimed to have found cellular groups, which in his opinion are to be considered as accessory parathyroids, in various places in the neck and in the upper pole of the thymus, yet he hedges by stating that these so-called accessory glands are always very small and only a minute part of the total parathyroid tissue and do

not seem to take part in any function of the gland. Moreover, he fails to present any plates showing the histological structure of these cell groups as supporting his contention that they are parathyroid tissue. It has been suggested that these small groups of cells could as well be thymic residues as accessory parathyroids. Later studies by Vincent and Jolly (8) and Thompson (9) sustain the earlier findings that the rat has but two parathyroid glands. I have carefully examined with a hand lens the neck and thymus of my operated animals and have yet to find any tissue that could be designated as parathyroid tissue outside of that found in close association with the thyroid.

In 1884 Schiff (10) described in detail the symptoms of tetany and disturbances of the nervous system following the removal of the thyroid gland from dogs, thus correlating them with similar occurrences observed after strumectomy in man previously reported from the clinical side by Weiss (11) and Reverdin (12). Since that time there have been many other descriptions of these phemonena, all phases of which I have seen in my operated rats, and which are now known to be due to the loss of the parathyroid secretion and not to a thyroid disturbance or the mechanical effects of the operation.

Inasmuch as the symptoms occurring after the removal of the parathyroids point unmistakably to an involvement of the nervous system in the final result, various workers have attempted to find histological evidences of alterations in the different parts of the nervous system of animals dying in parathyroid tetany. Much contradictory evidence is given. Awtokratow (13), Sanquirico and Canalis (14), de Quervain (15) and others could find no constant changes in the nerve elements sufficient to justify the opinion that anatomical alterations of cells or fibers are factors in the expression of parathyroid tetany. On the other hand Capobianco (16), Vassale and Donaggio (17), Maas (18), Lupó (19) and others report the presence of intensive degenerative changes in various parts of the nervous system. Unfortunately, however, but little agreement is found among the investigators as to the exact part of the nervous system attacked; though all agree that the tetany observed is due to an intoxication of the nervous system. These discrepancies are explicable from the fact that the specimens examined varied in the length of time they had been in the fixing fluid from a few days to a year or more; from the fact that the different workers failed to use the same method of fixation and staining; and from the fact that their specimens were taken from animals dying after thyreo-parathyroidectomy or parathyroidectomy at intervals from a few days to several weeks.

In 1912 Möllgaard (20), appreciating that a probable cause of these differences lay in the technique of fixation, made a histological comparison of the motor cells of the cord, the cells of the spinal ganglia, and the motor cortex of normal dogs and of dogs dying in parathyroid tetany. He used for his study sections cut from fresh frozen specimens. He found no changes in the motor cortex and no degenerative changes anywhere. He did find, however, that there were cells in the cord and spinal ganglia of the parathyroidectomised animals that differed from the normal in that no network could be brought out on staining. He therefore concluded that the cause of the parathyroid tetany and convulsions is an alteration of the physical condition of the nerve cells due to some toxic substance. This is supported from the physiological side by the experiments of Paton, Findlay and Watson (21) which they interpret as showing that the nervous symptoms are due to the condition of the central nervous system; that the cerebral arc is not directly involved; and that the electrical excitability of the peripheral nerves is increased when any marked decrease in parathyroid tissue has been produced.

Hence the evidence that the death of the parathyroidectomised animals is due to an immediate degenerative effect on the nerve elements of a toxic substance, either produced as a result of a perverted metabolism following the lack of the parathyroid secretion, or which is a normal product of neuro-muscular metabolism and is now uncompensated for by destruction or neutralization through the mediation of the parathyroid secretion, is quite generally negatived. This is particularly true from the fact that many of my parathyroidectomised rats exhibited tetany paralysis and convulsions within an hour and a half, and died in convulsions within three or four hours after the operation. The possibility of marked degenerative changes having occurred in the nervous system in vivo in this short period is rather dubious. This, however, does not exclude the possibility that through a long-continued action of the parathyroid deficiency there may be produced degenerative changes in the nerve elements by virtue of the repeated action of small amounts of the unknown constituent which in larger amounts produces acute tetany and death. In connection with the idea that these nerve disturbances are due to some toxic substance, attention should be called to the fact that although Paton and Findlay (22) consider that the idea of the parathyroid acting as "detoxicating some normal product of metabolism as hardly worthy of consideration" yet they base their theory of idiopathic and parathyroid tetany on the

development of some "guanidine-like body" in these conditions and state that "the parathyroids thus control the metabolism of guanidine in the body by preventing its development in undue amounts." This to my mind is a distinction without a difference.

From the reports of Cramer (23) and his collaborators, Gies (24) and his collaborators, Kojima (25) and Larson (27), it is evident that the rat can survive thyreo-parathyroidectomy or parathyroidectomy for some time without indications of an acute involvement of the nervous system, thus disproving Cristiani's (27) statement that complete thyroidectomy is always fatal. These observations lead one to suspect that either the nervous system of the survivors is different in its powers of resistance to the toxemia from that of those which die, or that the cause of the disturbances is not present in sufficient concentrations at any one time to produce acute symptoms.

My interest was attracted to this particular phase of the subject by the fact that although frequent deaths occurred from parathyroid tetany in my thyreo-parathyroidectomised rats, yet some of the operated animals did not die.

The general technique of removal of the thyroid apparatus was no different from that described by Cristiani (6) and others; but when the parathyroids were alone removed they were cut out of the thyroid with sharp, fine-pointed scissors. Hemorrhage was prevented by stopping off the superior thyroid arteries with small clamps made from ordinary paper-clips bent into appropriate shape and having the opposed ends flattened.

On examination it was found that the operated animals had come from two separate groups of rats, and that the greater percentage of survivals had occurred in the group that came from the so-called "Experimental Colony" of the Wistar Institute. We have here at the Wistar Institute two colonies of albino rats, among others, both of which are the descendants of the same original pair. One of these colonies, that which is called the Experimental colony, has been petted and gentled, while the other, the Standard stock, has had only that human contact incident to routine feeding and cage-cleaning. The behavior of the rats in this latter group is that of the ordinary laboratory animal. They are timid, apprehensive and high-strung. When picked up they are tense and resistant, frequently exhibiting their natural defensive instincts of fear and rage by biting. *The picture as a whole is one of constant high irritability and neuro-muscular tension.* It is of course not impossible that the high grade of emotional tension ever

present in the rats that have not been gentled contributes to the general picture of high tension through mediation of the adrenals.

The behavior of the gentled group is in marked contrast to that of the others. In these animals the defensive instincts have been repressed by the constant handling and petting. This gentling has been carried on for the last five generations. When the animals are picked up they are relaxed and yielding. They are not easily frightened. *They give a uniform picture of placidity. The threshold of the neuromuscular reactions to potentially disturbing stimuli is almost prohibitively high.*

We now turn to the actual experimentation carried out in order to determine whether or not there was a difference in the susceptibility of the animals from the two groups to the loss of the parathyroid secretion.

A total of 304 rats was operated. Both sexes were used in this study and the ages varied from 30 to 100 days. The majority of operations, however, were done on rats around 55 days old.

It has been noticed in the preliminary work that if the rats did not develop tetany or convulsions and die within 48 hours then they probably would continue to live for a considerable period thereafter. In fact the majority of survivals of the 48-hour period lived until they were 150 days old when they were killed for examination. Consequently it was evident that the fatalities from acute parathyroid tetany would usually occur in less than 48 hours. This period then was taken as the standard of probable survival.

Complete thyroidectomy, which included the removal of the parathyroids, was done on 90 rats from the Standard stock consisting of rats that had not been gentled. Out of these 79 per cent died in parathyroid tetany within the 48-hour period. During the same period 96 rats from the Experimental colony, the gentled stock, were similarly operated. The survivals from this series were many more. Eighty-seven per cent lived and but 13 per cent died. Among a series of rats from the Standard stock 28 were thyreo-parathyroidectomised in two stages. One-half of the thyroid apparatus was removed at the first operation and the second half was removed two weeks later. The mortality in this group was 68 per cent, or slightly lower than that of the first lot recorded. The lower mortality is explicable by the fact that during the interval some of the rats had become slightly less tense, though no special attempts had been made to gentle them.

In order to demonstrate that the results were not due to the loss of the thyroid but were due solely to the removal of the parathyroids, 51 rats from the Standard stock were parathyroidectomised. Within less than 48 hours 76 per cent of them had died. When 31 rats from the gentled stock were parathyroidectomised but 13 per cent of them died. The almost exact coincidence of these ratios with those of the thyreo-parathyroidectomised groups is conclusive evidence that we are here dealing with a parathyroid loss alone as the causative factor in the fatalities.

I next removed 28 animals born of the gentled stock from their mothers at weaning and put them, until they were 55 days old, under the same conditions of environment as the rats in the Standard stock. They were not gentled. At the same time a like number of rats from the Standard stock were removed from their mothers at weaning and set aside under their usual environmental conditions for thyreo-parathyroidectomy at 55 days of age as controls. The usual 78 per cent of fatalities occurred in the Standard rats. However, much to my surprise, when the progeny of the gentled rats were brought to me they were almost as tame as their parents and when completely thyroidectomised but 14 per cent of them died. This rather indicates that the gentling process for three generations had resulted in the elimination of the defensive reactions of the young with a consequent stabilization of the nervous system and lowered tension which had been passed along.

A final study was then made in which one-half of each of several litters of rats from the Standard stock were placed in the Experimental colony at weaning and gentled, while the other half was to have been kept under the usual conditions of Standard room care and not gentled. As controls the same number of animals from several litters of the gentled stock were similarly split, one-half of which was put under the Standard stock conditions and the other half kept and gentled as usual. When this series was brought to the laboratory for operation at the age of 55 days they all, those from the Standard stock as well as those from the gentled stock, appeared tame, non-resistant and relaxed. Acute tetany did not appear within the 48-hour period in any of these rats after the loss of the parathyroid secretion by operation. This definitely showed that the stability of the nervous system is a strong factor in the resistance of albino rats to the loss of the parathyroid secretion. A study of the records of the sex, body weight and body length of all the lots operated showed that these factors could not be considered as playing any significant rôle in the final results. Nor could any dietary

differences of either qualitative or quantitative nature be invoked as contributing causes. The only observable differences in the two groups of operated animals are their nervous reactions to the environment as already described. That such differences have been shown to exert an influence on metabolic stability has already been indicated (28). That they should result in such marked differences in dependency on the parathyroid secretion is remarkable and affords conclusive evidence that a condition of nervous stability is conducive to a greater resistance to the loss of the parathyroid secretion than is a condition of high-strung irritability.

In view of the striking differences obtained a brief attempt at interpretation seems justified, based on related data from other sources. It would be a gratuitous assumption to consider that the differences in mortality in the two groups were due to differences in grade of parathyroid secretion, since we have no direct indication of this latter possibility. Yet if the condition of hypertension in the rats that had not been gentled is to be considered as a result of a diminished parathyroid secretion, the condition of stability shown in the gentled group should *pari passu* be considered as due to a relatively greater parathyroid secretion. If such is the case this would imply a natural or acquired greater dependency on such secretion and hence more serious defection on its removal. This we have seen is not the case.

Now it is a well-known fact that excitability tends to the production of a condition of heightened muscular tone, while calmness or lack of emotional strain results in a lower tension. This simply means that the metabolic changes taking place incident to the maintenance of muscle tone are of a lower order of magnitude in gentled animals than they are in rats that have not been gentled. Such being the case, let us assume as a working basis that the normal function of the parathyroid is to prevent the accumulation of or to cause the catalytic destruction of any tetany-producing substance such as ammonia, which MacCallum and Voegtlin (29) have shown to be excreted in more than normal amounts in parathyroid tetany; which Carlson and Jacobson (30) showed to cause tetany; and which Tashiro (31) has demonstrated to be a product of the tetanized nerve-muscle preparation; or of guanidine, which Burns and Sharpe (32) have found to be increased in the blood and urine of parathyroidectomised dogs, and which Paton and Findlay (22) have shown to produce symptoms identical with those produced by parathyroid tetany. Then in either case, whether these substances are abnormal by-products from the metabolism of muscle tone due to the lack of the regulating effect of the parathyroid secretion

on that phase of muscle metabolism, or whether they are normal products of this reaction and are not neutralized or destroyed because of the lack of the catalyzing agent produced by the parathyroids, the explanation of the high mortality in the group of rats of high nervous tension can be based on the idea of an inherently greater tendency to the formation of the toxic compounds than is found in those rats of low muscle tone. When the parathyroids are removed from the latter type of animal the decrease in the catalytic effect is less destructive because less of the toxic compounds are normally produced and the animal continues to live because the body as a whole is able in some way to dispose of this smaller amount on the basis of the same general principle Kendall (33) has evolved for the vitality expressed in athyroid conditions.

SUMMARY AND CONCLUSIONS

A study is reported of the relative mortality after thyreo-parathyroidectomy and parathyroidectomy of two groups of albino rats, differing only in the stability of their nervous systems as evidenced by excitability and a high-tension reaction to the environment in the one group, designated as Standard stock, or rats that had not been gentled; and by a condition of relaxation and high threshold toward potentially disturbing stimuli in the other or gentled group. In all, 304 rats were operated. Complete thyroidectomy resulted in a mortality by acute parathyroid tetany of 79 per cent of the Standard stock rats. The same operation in the gentled rats gave but a 13 per cent mortality. The same ratios were found to hold when parathyroidectomy alone was done. Gentled rats of the third generation when put from weaning under conditions of identical environment as those obtaining for the Standard group still showed but a 14 per cent mortality. Standard stock rats when gentled had their mortality rate reduced to zero in the relatively small series studied. No sex, size, weight or dietary variations can be used as explaining the differences in mortality of the two groups. For reasons explained in the text, 48 hours was taken as the standard period of survival in the comparisons.

It is therefore concluded that the condition of stability of the nervous system induced in albino rats by gentling and petting produces in them a marked resistance to the loss of the parathyroid secretion, which in excitable rats normally results in death from acute parathyroid tetany in less than 48 hours.

This is interpreted on the basis of our present knowledge to be possibly due to an inherently lesser production in the gentled rats of

certain known tetany-producing substances such as ammonia or guanidine as by-products of that phase of muscle metabolism concerned with muscle tone. Whether or not these toxic substances accumulate in the operated rats that have not been gentled because of an abnormal metabolism due to the loss of a regulating influence exerted by the parathyroids, or whether they are normal products of muscle catabolism and accumulate because they are not neutralized or destroyed by the catalytic action of the parathyroid secretion is a question calling for further study.

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STUDIES IN NUTRITION

VI. The Nutritive Value of the Proteins of the Lima Bean, Phaseolus Lunatus

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The fact that the proteins of the navy bean, *Phaseolus vulgaris*, not only are deficient in cystine but also require cooking for a short time before they become biologically available has been shown in a recent publication from this laboratory (1). This suggested a line of attack upon the problem of the nutritive value of the closely related lima bean, *Phaseolus lunatus*. At the time that the paper on the navy bean was published the question as to what effect cooking had upon the proteins was left in abeyance. It has since been shown (2) that the difference due to cooking is to be explained, at least in part, to an increase in digestibility.

In the present paper it is shown that the lima bean when cooked, dried, supplemented with cystine, and fed to albino rats as the sole source of protein in an otherwise complete diet produced normal growth. The animals fed a similar diet without the addition of cystine merely maintained their weight. Those receiving a diet of raw lima bean meal, however, made no growth, even if cystine was added to complete the diet.

Experiments with raw and cooked lima bean meal. A diet was prepared which contained 75 parts of raw lima bean meal, 11 parts of lard, 10 parts of butter fat and 4 parts of an inorganic salt mixture.[1] A similar diet was made with lima bean meal which had been cooked and dried. Both of these diets contained 15 per cent of protein. The rats fed either of the diets made no growth, but were able to maintain their weight. The growth curves are shown on chart 1.

[1] The composition of the salt mixture used in the diets described in this paper is the same as that used by Osborne and Mendel (3) in their experimental diets.

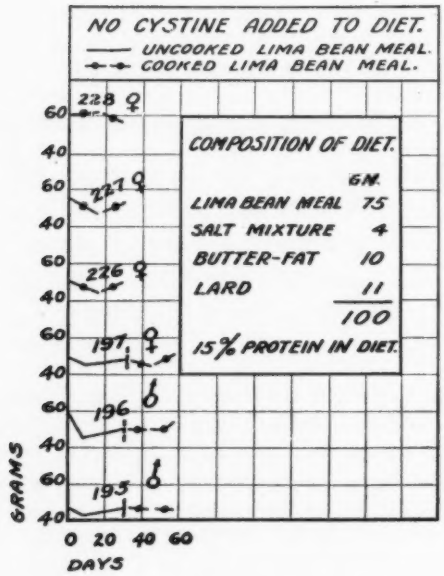

Chart 1

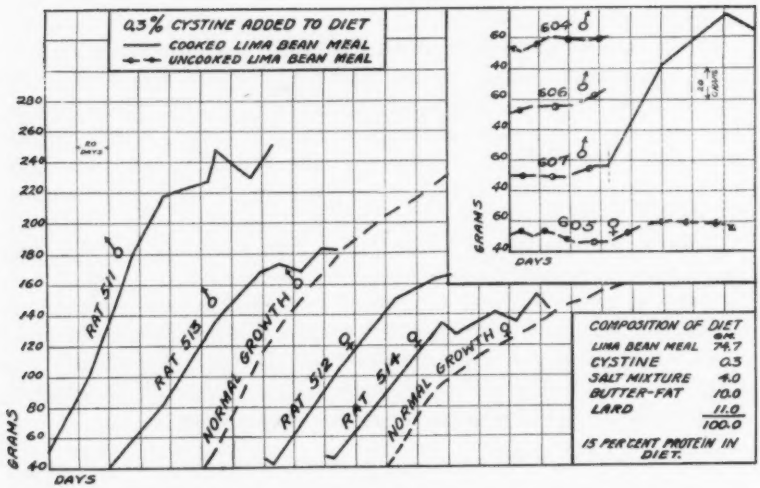

Chart 2

Experiments with raw and cooked lima bean meal supplemented with cystine. Two diets were prepared, one from cooked and the other from uncooked lima bean meal, to both of which 0.3 per cent of cystine was added. To complete these diets, each of which contained 15 per cent of protein, butter fat, lard and an inorganic salt mixture were added. The animals receiving the cooked lima bean meal supplemented with cystine grew normally, while those fed the raw lima bean meal and cystine diet merely maintained their weight. These experiments indicate that the lima bean meal, after it had been cooked and dried, contained sufficient water-soluble vitamine. Rat 607 ♂ gained very little in weight the first 65 days, during which it received the raw lima bean diet supplemented with 0.3 per cent cystine. At the end of this period it was given a similar diet with the exception that the lima bean meal had been cooked. The animal then at once resumed the normal rate of growth. The composition of the diets and the growth curves are recorded on chart 2.

SUMMARY

A diet of cooked lima bean meal supplemented with 0.3 per cent of cystine, together with the other necessary non-protein dietary ingredients, furnished adequate protein for the normal growth of albino rats. A similar diet, to which no cystine was added, merely maintained the weight of the experimental animals. Growth did not occur if the diet consisted of either raw or cooked lima bean meal which was not supplemented with cystine although the other non-protein dietary factors were added.

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STUDIES IN NUTRITION

VII. The Nutritive Value of the Proteins of the Adsuki Bean, Phaseolus Angularis

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The adsuki bean, *Phaseolus angularis*, has long been used as food by the people of the Orient. Its varieties are very numerous and are distinguished principally by their period of maturity, together with the color of seeds and pods. The seeds are subcylindric with ends a little flattened and slightly longer than broad and the embryo in all cases nearly white and brittle in consistency.[1]

Nutritive studies with the navy and lima beans, which belong to the same botanical genus, have already been published from this laboratory (1), (2). The proteins of these two seeds lacked two factors before they became available for the normal nutrition of albino rats. First, a deficiency of cystine, which was corrected by the addition of 0.3 per cent of this amino acid to a diet adequate in other respects. Second, cooking, without which growth could not be obtained. In the case of the adsuki bean, however, while it was found that its proteins were deficient in cystine, cooking was not required.

Experiments were made with the isolated adsuki globulin as well as with raw and cooked adsuki bean meal. When the isolated protein,[2] together with the other non-protein dietary essentials, was fed to albino rats they grew at about two-thirds of the normal rate. If, however,

[1] The beans used in the experiments described in this paper were furnished by the Bureau of Plant Industry, United States Department of Agriculture, and were the maroon variety. A complete description of the adsuki bean is given by C. V. Piper and W. J. Morse in U. S. Dept. Agric. Bulletin 119, 1914.

[2] The adsuki protein was prepared by precipitating a clarified 1 per cent sodium chloride extract of adsuki bean meal in 5 volumes of distilled water. The protein thus obtained was washed until free from chlorides and the water removed with absolute alcohol and absolute ether. Unpublished experiments by Chas. E. F. Gersdorff of this laboratory indicate that the adsuki protein so prepared is a mixture containing two globulins differing in their sulfur content.

the protein was supplemented with cystine, the rate of growth was markedly increased.

A diet of either raw or cooked adsuki bean meal and the other necessary dietary ingredients enabled albino rats to grow at only about one-third to two-thirds of the normal rate. If, however, 0.34 per cent of cystine was first added to either the raw or cooked bean meal, the rats grew at the normal rate. The growth obtained with the raw adsuki bean meal supplemented with cystine was surprising in view of the fact that neither the navy nor the lima bean produced growth, even when this factor was added.

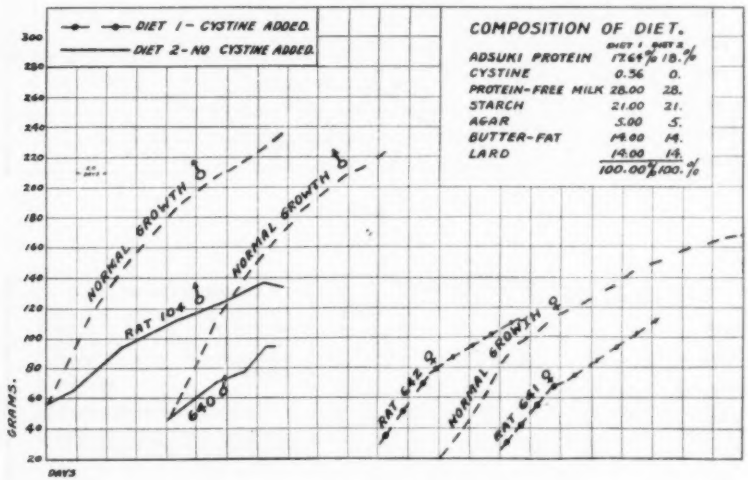

Chart 1

Experiments with the isolated adsuki protein. Two diets were prepared from the adsuki protein, in one of which the protein was supplemented with 2 per cent of cystine. The water-soluble vitamine and inorganic salts were furnished by protein-free milk, while butter fat gave the necessary fat-soluble vitamines, and the diet was made adequate in other respects. Rats 641 ♀ and 642 ♀ were placed on diet 1, which contained cystine. These animals grew at almost the normal rate. Diet 2, which had not been supplemented with cystine, was fed to rats 104 ♂ and 640 ♂ and the rate of growth was from one-third to one-half of the normal. The composition of the diets and the growth curves are recorded on chart 1.

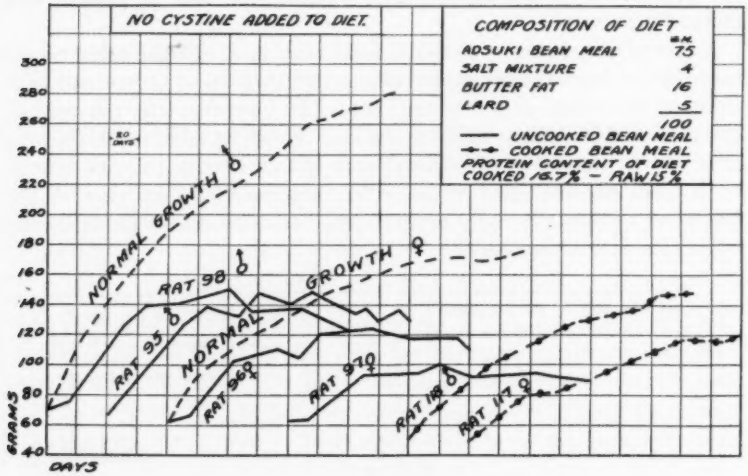

Chart 2

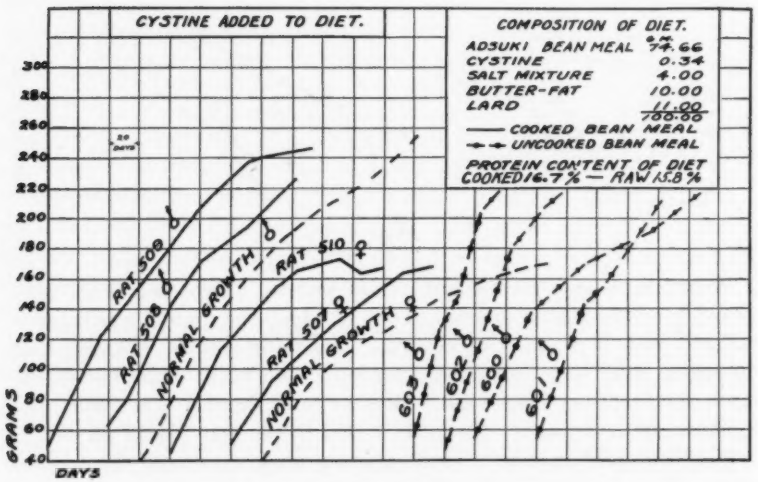

Chart 3

Growths experiments with raw and cooked adsuki bean meal. Diets were prepared from both the raw and cooked bean meal, to which was added an adequate inorganic salt mixture,[3] butter fat and lard. These diets enabled the rats to grow at only from one-third to two-thirds of the normal rate. The composition of the diet and the growth curves are shown on chart 2.

Growth experiments with raw and cooked adsuki bean meal, supplemented with 0.34 per cent of cystine. Rats grew at the normal rate when fed a diet similar to that described above, except that 0.34 per cent of cystine replaced an equivalent quantity of the bean meal. It is interesting to note that the raw bean meal, when supplemented with cystine, was as efficient in producing normal growth as the cooked meal. These results are shown graphically on chart 3.

TABLE 1

*Average gain of body weight per gram of ingested protein. 4-week period**

DIET	COOKED ADSUKI BEAN	COOKED LIMA BEAN	COOKED NAVY BEAN	COOKED PHASEOLIN†	RAW ADSUKI BEAN	RAW LIMA BEAN
Gain per gram of protein ingested (*grams*)	1.5	1.2	1.5	1.5	1.7	0.16
Protein in diet (*per cent*)	16.7	15	18	20	15.8	15

* Cystine was added to all diets.

† Isolated navy bean protein.

Relative efficiency of the proteins of the genus Phaseolus. The weight of food ingested was determined each week and compared with the rate of growth. The data given in the following table show that there was very little difference in the gain in weight made by the animals for each gram of protein eaten when the beans were cooked and properly supplemented with cystine. The raw proteins of the adsuki bean were as efficient as the cooked for normal growth when cystine was added, while the raw proteins of the navy and lima beans, similarly supplemented, did not promote growth. These figures are given in table 1.

[3] For the composition of the salt mixture see Osborne and Mendel, Journ. Biol. Chem., 1917, xxxii, 374.

SUMMARY

Raw or cooked adsuki bean meal supplemented with cystine furnished adequate protein and water-soluble vitamine for normal growth. Similar diets without the addition of cystine enabled the albino rats to grow at only one-third to two-thirds of the normal rate. Comparable results were obtained with the isolated adsuki bean globulin.

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THE EPINEPHRIN OUTPUT ESTIMATED BY COLLECTING THE ADRENAL BLOOD WITHOUT OPENING THE ABDOMEN

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It has been assumed by some writers that when blood from the adrenals is collected in the ordinary way through an abdominal incision, the secretion of epinephrin may be artificially increased by the experimental conditions, and that the rate of output estimated under these conditions may therefore be too high. No experimental evidence in support of this assumption has been brought forward. We have nevertheless judged it worth while to compare the output estimated on blood obtained by the abdominal operation, with that estimated on blood obtained by an extraperitoneal operation. It is possible, without opening the peritoneum, to prepare the left lumbo-adrenal vein in dogs through a lumbar incision, so as to collect the blood flowing from the adrenal without obstruction.

A lumbar incision is made, beginning just below the last rib and extending downward about 2½ to 3 inches. The muscles are either cut or the fibers separated until the peritoneum is seen. With the aid of a gauze sponge the peritoneum is carefully separated downward (and backward) from the adjacent tissues, exposing the adrenal gland with its vein entering the cava, or, as is not infrequently found, the renal vein, especially in dogs. During this procedure it is necessary to exercise caution not to tear the lumbar vein or one of the small veins entering it. A loose ligature is placed around the adrenal vein between the gland and the cava. A cannula is inserted into the lumbar end of the lumbo-adrenal vein, after tying small veins entering it, and the course of the adrenal blood directed through the cannula by occluding the adrenal vein at the cava (or renal vein). This is accomplished by traction on the loose ligature or by clipping the vein, the ligature serving as a guide to the adrenal vein.

As will be seen in the experiments quoted, the outputs determined on the blood collected extraperitoneally were within the usual range of the outputs estimated in etherized dogs with blood collected after

opening the abdomen. Further, when the output was calculated on one and the same animal, first, with adrenal blood collected extraperitoneally and then with adrenal blood collected after the abdomen was opened, no clear difference could be made out, within the limits of error of the method of estimation.

In the first experiment to be cited (dog 355) blood was obtained from the left adrenal through a lumbar incision. The abdomen was then opened and blood collected from both adrenals.

Condensed protocol; dog 355; female; weight 17.85 kgm.

11:25 a.m. to 12:00 m. Under morphine and ether, *via* extraperitoneal route (lumbar incision), inserted cannula into lumbar end of left lumbo-adrenal vein. By occluding adrenal vein at the junction with the cava, with a small clip, collected blood from the left adrenal.

12:01 p.m. First specimen, 2.75 grams in 30 seconds (5.5 grams per minute).

12:01½ p.m. Second specimen, 10.9 grams in 3 minutes (3.6 grams per minute). Removed clip from left adrenal vein.

12:10 to 12:35 p.m. Abdomen opened, cava pocket completed (coeliac axis and superior mesenteric arteries were tied, in addition to the blood vessels usually tied). Collected blood from both adrenals.

12:35½ p.m. Third specimen, 10.7 grams in 30 seconds (21.4 grams per minute).

12:36 p.m. Fourth specimen, 21.6 grams in one minute. Obtained indifferent (venous) blood specimen. Combined weight of adrenals, 2.3 grams.

The second adrenal specimen, collected from the left adrenal extraperitoneally, caused a much greater inhibition of the intestine segment than the fourth specimen, collected from both adrenals after opening the abdomen, corresponding to the greatly increased flow in the fourth due to the increased blood pressure caused by tying the coeliac axis, superior mesenteric artery and abdominal aorta (fig. 1, observations 48 and 54).

The assay showed that the second specimen was stronger than 1:7,000,000, stronger than 1:5,700,000, somewhat weaker than 1:4,300,000, decidedly weaker than 1:3,500,000 (fig. 1, observations 48 and 50, and other observations not reproduced). The second specimen was assayed at 1:5,000,000, corresponding to an output of 0.0007 mgm. per minute, or 0.00004 mgm. per kilogram per minute for the left adrenal alone. On the assumption that the output is the same for each adrenal, this would give 0.00008 mgm. per kilogram per minute for the two adrenals.

The fourth specimen, collected from both adrenals after opening the abdomen, was found to be decidedly weaker than 1:10,000,000 adrena-

lin, weaker than 1:13,000,000 (confirmed by several sets of observations), weaker than 1:14,300,000, not much different from 1:17,000,000. Taking it at 1:17,000,000, we get 0.0013 mgm. per minute as the output from both adrenals, i.e., 0.00007 mgm. per kilogram of body weight per minute. The output per kilogram of body weight is less than the average, but still within the normal range. We do not know whether the average output per kilogram is the same for large as for small dogs, but the matter is of no importance for our present question.

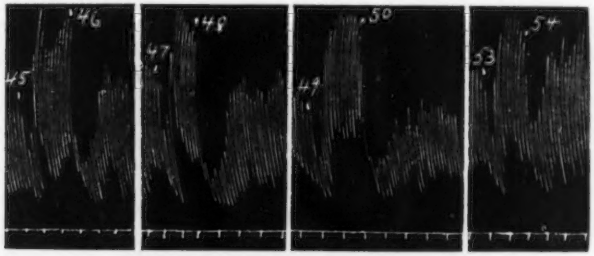

Fig. 1. Intestine tracings. Bloods from dog 355. At *45*, *47*, *49* and *53*, Ringer was replaced by indifferent (jugular) blood and this, at *46*, by indifferent blood to which was added adrenalin to make a concentration of 1:7,000,000; at *48*, by the second adrenal blood specimen; at *50*, by indifferent blood to which was added adrenalin to make a concentration of 1:3,500,000; at *54*, by the fourth adrenal specimen. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). Time trace in half-minutes. Reduced to one-half.

In the next experiment (dog 487) the left lumbo-adrenal vein was permanently tied off near the cava after specimens of the adrenal blood (first, second and third) had been collected. The abdomen was then opened and blood collected from the remaining (right) adrenal in the usual way.

Condensed protocol; dog 487; male; weight, 13.78 kgm.

10:00 to 10:30 a.m. Under ether, *via* extraperitoneal route (lumbar incision), inserted cannula into lumbar end of left lumbo-adrenal vein. By occluding adrenal vein at the junction with the cava, collected blood from left adrenal.

10:31½ a.m. First specimen, 2.1 grams in 30 seconds (4.2 grams per minute).

10:32 a.m. Second specimen, 10.9 grams in 3 minutes (3.6 grams per minute).

10:35 a.m. Third specimen, 11.0 grams in 3 minutes (3.7 grams per minute).

10:40 to 11:03 a.m. Tied left adrenal vein. Abdomen opened and cava pocket completed in usual manner, the coeliac axis and superior mesenteric artery not being tied. Collected blood from right adrenal.
11:04 a.m. Fourth specimen, 5.0 grams in one minute.
11:05 a.m. Fifth specimen, 15.2 grams in 3 minutes (5.1 grams per minute). Obtained indifferent (arterial) blood specimen. Combined weight of adrenals, 1.7 gram.

The second and third adrenal specimens had about the same concentration of epinephrin. They were shown to be much stronger than 1:5,300,000, stronger than 1:2,700,000, decidedly weaker than

Fig. 2. Intestine tracings. Bloods from dog 487. At *7* and *9* Ringer was replaced by indifferent (arterial) blood and this, at *8*, by indifferent blood to which was added adrenalin to make a concentration of 1:1,325,000; at *10*, by the third adrenal blood specimen (collected from left adrenal by extraperitoneal route). All the bloods were diluted with three volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

1:1,350,000 (fig. 2, confirmed by several other observations). As the reaction when the blood was simply diluted with three volumes of Ringer's solution was too strong for an exact assay, the third specimen was now diluted with one volume of indifferent blood before dilution with Ringer's solution. In this way the third specimen was shown to be somewhat weaker than 1:2,000,000, stronger than 1:2,650,000 (fig. 3, confirmed by other observations not reproduced). It was assayed at 1:2,300,000, corresponding to an output of 0.0016 mgm.

per minute, or 0.00012 mgm. per kilogram of body weight per minute for the left adrenal alone. This is fully equal to the average output for dogs, under the conditions of our experiments (1), when the adrenal blood is collected after opening the abdomen (average 0.00022 mgm. per kilogram per minute for the two adrenals).

The fifth adrenal specimen, collected from the right adrenal after opening the abdomen, had a concentration of epinephrin not very different from that of the second and third specimens, although the blood

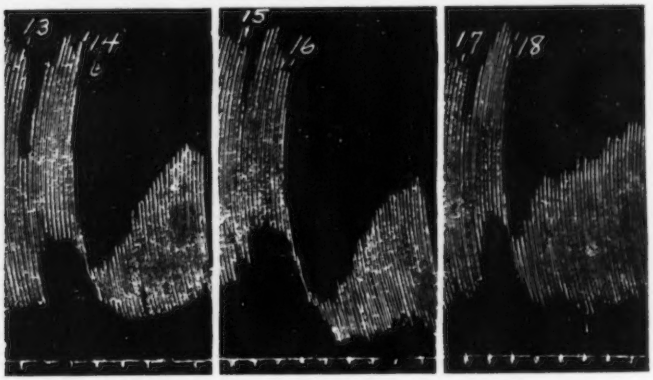

Fig. 3. Intestine tracings. Bloods from dog 487. At *13*, *15* and *17*, Ringer was replaced by indifferent (arterial) blood and this, at *14*, by the third adrenal blood specimen (collected from left adrenal by extraperitoneal route) diluted with one volume of indifferent blood; at *16*, by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000; at *18*, by indifferent blood to which was added adrenalin to make a concentration of 1:5,300,000. All the bloods were diluted with three volumes Ringer (the adrenal blood after adding the indifferent blood, and the adrenalin bloods after adding the adrenalin). Reduced to one-half.

flow was somewhat greater, as the blood pressure had been raised by tying the abdominal aorta when the abdomen was opened. The fifth specimen was shown to be decidedly stronger than 1:2,650,000, weaker than 1:1,325,000 (fig. 4, confirmed by other observations not reproduced). The assay was now continued with the fifth specimen diluted with one volume of ind fferent blood, and the fifth specimen was found to be somewhat stronger than 1:2,650,000, but decidedly weaker than 1:2,000,000 (fig. 5, observations 32 to 36). Qualitatively it was shown

again that there was no great difference in concentration between the third and the fifth specimens (fig. 5, observations 42 and 44), whereas the first (preliminary) specimen was much weaker than the second (fig. 5, observations 38 and 40). This indicates that manipulation in the insertion of the cannula by the lumbar route was not in any way responsible for the output of epinephrin in the second and third speci-

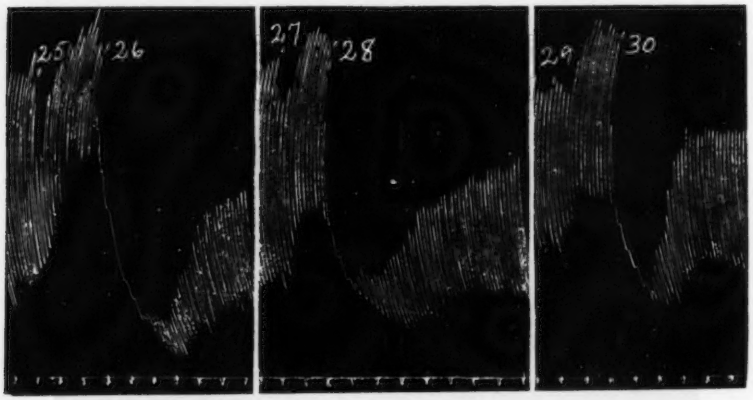

Fig. 4. Intestine tracings. Bloods from dog 487. At *25*, *27* and *29* Ringer was replaced by indifferent (arterial) blood, and this, at *26*, by indifferent blood to which was added adrenalin to make a concentration of 1:1,325,000; at *28*, by the fifth adrenal blood specimen (collected from the right adrenal by "cava pocket"); at *30*, by indifferent blood to which was added adrenalin to make a concentration of 1:2,650,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

mens. The fifth specimen was taken at 1:2,500,000, corresponding to an output of 0.002 mgm. per minute or 0.00014 mgm. per kilogram per minute for the right adrenal, practically the same as the output obtained for the left adrenal with extraperitoneal collection of the blood. It is obvious that the opening of the abdomen could not have sensibly increased the output of epinephrin from the right adrenal.

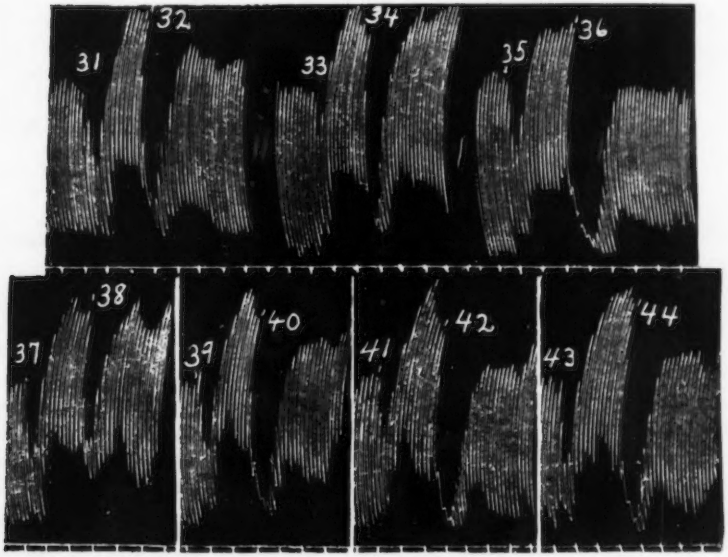

Fig. 5. Intestine tracings. Bloods from dog 487. At *31*, *33*, *35*, *37*, *39*, *41* and *43* Ringer was replaced by indifferent (arterial) blood, and this, at *32*, by the fifth adrenal blood specimen (collected from the right adrenal by "cava pocket"), diluted with one volume indifferent blood; at *34*, by indifferent blood to which was added adrenalin to make a concentration of 1:5,300,000; at *36*, by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000; at *38*, by the first adrenal specimen (collected from left adrenal by extraperitoneal route), diluted with one volume indifferent blood; at *40*, by the second adrenal specimen (collected from the left adrenal by extraperitoneal route), diluted with one volume indifferent blood; at *42*, by the third adrenal specimen (collected from the left adrenal by extraperitoneal route), diluted with one volume indifferent blood; at *44*, by the fifth adrenal specimen diluted with one volume indifferent blood. All the bloods were diluted with three volumes Ringer (the adrenal bloods after adding the indifferent blood, and the adrenalin bloods after adding the adrenalin). Reduced to one-half.

SUMMARY

The rate of epinephrin output is approximately the same whether it is estimated on adrenal vein blood collected without opening the abdomen or after opening the abdomen.

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POST-OPERATIVE DEPLETION OF THE EPINEPHRIN STORE OF THE ADRENALS

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The progressive diminution in the epinephrin store of the adrenals, which goes on at least for several hours after an operation when the innervation of the glands has not been interfered with, has been studied by us in a previous paper (1). It might be associated solely with the action of the anesthetic since Elliott (2) has shown that general anesthetics, such as ether, chloroform or urethane, cause depletion of the store of an adrenal whose nerves are intact, as compared with that of its previously denervated fellow. Or the depletion might be due to the effects of the trauma as well as to the anesthetic. In either case the depletion could be caused by an increase in the rate of output of epinephrin through the epinephrin-secretory nervous mechanism, without change in the rate of formation of epinephrin, or to interference with the formation of epinephrin, while the liberation went on without change from the innervated gland, or finally to any disturbance of the normal balance between production and output in favor of the latter. That trauma as such, or at least the trauma associated with abdominal operations for the study of the epinephrin output, does not influence the output in an important degree, is indicated by the observations in the immediately preceding paper (3), in which it is shown that the epinephrin output in dogs is, within the limits of error of our experimental methods, the same when estimated upon adrenal blood collected extraperitoneally through a lumbar incision, and upon adrenal blood collected after opening the abdomen. There remains the possibility that the trauma might interfere with the filling up of the epinephrin store. The experiments summarized in table 1 show, however, that when one adrenal is removed under local anesthesia (ethyl chloride), the wound sutured, and the other adrenal removed 5 to 7 hours later, after killing the animal suddenly, there is generally no depletion

of the epinephrin store of the second adrenal. In 9 out of 15 rabbits the store of the second adrenal, as estimated by the method of Folin, Cannon and Denis (4) was precisely equal to that of the first. In one rabbit the store of the second adrenal was a little greater than that of the first. In two of the remaining rabbits (368 and 400) the difference

TABLE 1

Normal rabbits

NUMBER OF ANIMAL	BODY WEIGHT	ADRENAL WEIGHT		ADRENAL WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WEIGHT	EPINEPHRIN		EPINEPHRIN PER GRAM OF FIRST ADRENAL REMOVED	EPINEPHRIN PER KILOGRAM BODY WEIGHT	RATIO OF EPINEPHRIN IN FIRST TO THAT IN LAST REMOVED ADRENAL	INTERVAL BETWEEN REMOVAL OF ADRENALS
		Left	Right		Left	Right				
	kgm.	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*		*hours*
359	2.68	467	430	334	0.45	0.45	0.96	0.34	1.00	6½
364	1.48	72	70	96	0.10	0.10	1.39	0.13	1.00	6
367	2.09	200	170	177	0.38	0.38	1.90	0.36	1.00	4¾
368	2.06	160	120	136	0.12	0.15	1.25	0.14	1.25	5¾
369	2.45	300	327	256	0.22	0.24	0.73	0.19	0.92	7½
371	2.22	130	116	110	0.15	0.15	1.15	0.14	1.00	6¾
373	2.06	185	170	172	0.16	0.16	0.86	0.16	1.00	7
375	2.64	285	256	206	0.27	0.18	0.95	0.20	1.50	6
397	3.01	241	200	146	0.21	0.15	0.87	0.14	1.40	6½
400	2.43	206	194	164	0.15	0.12	0.70	0.12	1.25	6
401	2.69	228	212	163	0.20	0.20	0.88	0.15	1.00	5¾
402	2.08	250	246	238	0.13	0.13	0.52	0.13	1.00	5¾
403	2.52	169	176	137	0.18	0.18	1.06	0.14	1.00	7
404	2.57	200	188	151	0.22	0.16	1.10	0.17	1.37	6½
405	2.94	236	221	155	0.20	0.20	0.85	0.14	1.00	6¼
Average..	2.39	222	206	176			1.01	0.18	1.11	

Rabbits 368, 369, 371 and 403 were females, the last in early pregnancy. The left adrenal was removed first in all the animals in the table except 368. The operation was extraperitoneal in 359, 368, 369 and 373; in all the others the peritoneum was opened.

between the stores of the first and second adrenals was not greater than may be found in two adrenals removed simultaneously (1). In 3 of the 15 rabbits there seems to have been a genuine, moderate depletion of the second adrenal as compared with the first. The average of the ratios of the epinephrin store of the first to that of the second

adrenal removed for the whole 15 animals is, however, only 1.11:1. Or putting it in another way, the sum of the epinephrin stores in the adrenals first excised is 3.17 mgm., and in the adrenals excised later 2.92 mgm., giving a ratio of 1.08:1.

In one cat, a young female weighing 1.49 kgm., the left adrenal was removed under ethyl chloride anesthesia by an extraperitoneal operation. It weighed 0.16 gm. and contained 0.20 mgm. epinephrin. The cat was killed 6½ hours later and the right adrenal removed. It weighed 0.182 gm. and contained 0.18 mgm. epinephrin, showing no definite post-operative depletion.

So far, then, as these results go, the influence of trauma on the post-operative depletion, appears to be very slight and inconstant.

A second series of observations was made in the same way on 25 rabbits from which the thyroids and the whole, or the greater portion of the parathyroids had been removed. These rabbits were kindly handed over to us by Dr. David Marine, who had used them in work carried on by himself and Dr. O. T. Manley, and desired to sacrifice them for autopsy.

In most of the rabbits the thyroid operation had been performed 7 or 8 weeks before they were given to us. In rabbits 428 and 429 the thyroids and parathyroids were excised 15 weeks, in rabbits 414 and 426 only 5 weeks before we made our observations on the adrenals. In rabbits 480, 481, 482 and 483 the thyroids were removed more than a year before we got them. In all cases most of the right and left lobes (with the superior parathyroids) was excised, also the whole of the right inferior parathyroid and ½ to $\frac{9}{10}$ of the left inferior parathyroid, or sometimes the whole. In rabbit 360 it is noted that all the left inferior parathyroid was removed, and in 389 that ¾ of it was removed and the blood supply of the stump probably interfered with. The majority of the animals received calcium chloride for some time, and tetany was only observed in one or two cases. When calcium chloride was given it was discontinued at least a month before the animals were handed over to us. Thyroid homeo-transplantation under the skin of the abdomen was done in many of the animals, but the grafts were practically always found negative. The last examination of the grafts, under ether, was about a month to 6 weeks before we got the animals, but in rabbits 414, 425, 426, 428 and 429 the last etherization was only 6 to 9 days before they were handed over to us. It is noted that one of the animals (414) had been repeatedly injected with sheep's erythrocytes, the last injection being made one month before the thyroids and the whole of the parathyroids were removed.

Of the 25 rabbits in table 2, 16 gave practical equality of epinephrin store in the two adrenals, or a difference not clearly surpassing the range of the ordinary variation. Three animals are included in this group in which the store of the adrenal last removed was somewhat

TABLE 2

Thyro-parathyroidectomised rabbits

NUMBER OF ANIMAL	BODY WEIGHT	ADRENAL WEIGHT		ADRENAL WEIGHT IN MILLIGRAMS PER KILOGRAM OF BODY WEIGHT	EPINEPHRIN		EPINEPHRIN PER GRAM OF ADRENAL FIRST REMOVED	EPINEPHRIN PER KILOGRAM BODY WEIGHT	RATIO OF EPINEPHRIN IN FIRST TO THAT IN LAST REMOVED ADRENAL	INTERVAL BETWEEN REMOVAL OF ADRENALS
		Left	Right		Left	Right				
	kgm.	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*		*hours*
360	2.32	350	384	316	0.58	0.42	1.66	0.50	1.38	**6**
362	2.08	408	392	385	0.49	0.40	1.20	0.47	1.22	6½
363	2.14	206	170	176	0.08	0.13*	0.76	0.12	1.62	**6**
365	2.03	261	250	252	0.23	0.27*	1.08	0.27	1.17	5½
366	2.12	200	200	188	0.18	0.18	0.90	0.17	1.00	4¾
370	1.60	204	190	246	0.13	0.09	0.64	0.16	1.44	7
372	1.90	140	190	174	0.13	0.095	0.93	0.14	1.36	7¼
374	2.08	420	400	394	0.32	0.17	0.76	0.38	1.88	6½
389	2.26	992	878	827	1.12	0.76	1.13	0.99	1.47	7
390	2.26	488	436	409	0.72	0.72	1.47	0.64	1.00	6½
391	1.79	280	292	320	0.20	0.13	0.70	0.22	1.54	6¼
396	2.15	280	252	247	0.31	0.18	1.11	0.29	1.72	7¼
398	1.75	435	356	452	0.45	0.24	1.03	0.51	1.87	6¼
412	2.26	247	228	210	0.20	0.18*	0.79	0.17	0.90	6½
413	1.92	258	222	250	0.24	0.26*	1.17	0.27	1.08	6½
414	2.61	326	306	242	0.36	0.36*	1.17	0.28	1.00	6
426	2.32	252	241	212	0.26	0.24	1.03	0.22	1.08	7
427	1.51	186	190	249	0.11	0.06	0.54	0.15	1.83	6½
428	2.47	296	280	233	0.30	0.26	1.01	0.24	1.15	8
429	2.37	291	278	240	0.26	0.22	0.89	0.22	1.18	7½
430	1.07	108	96	190	0.11	0.09	1.02	0.20	1.22	7
480	1.80	198	212	228	0.18	0.15*	0.71	0.18	0.83	6½
481	2.31	290	311	260	0.27	0.24	0.93	0.23	1.12	6½
482	3.70	410	490	243	0.36	0.42	0.88	0.21	0.85	6¼
483	2.57	300	300	233	0.21	0.21	0.70	0.16	1.00	6¼
Average..	2.13	313	302	287			0.97	0.29	1.27	

* Right adrenal first removed; in all the others the left adrenal was first removed. In rabbits 362, 365, 366, 372, 413, 426, 427, 428, 429, 430, 480 and 481 the adrenals were removed without opening the peritoneum; in the rest the peritoneum was opened. Rabbits 360, 362, 363, 365, 366, 370, 426, 427, 430, 480, 481 and 483 were females; the rest males. Rabbits 414 and 426 were castrated one and four weeks respectively before thyro-parathyroidectomy, rabbits 428 and 429 seven weeks after thyroid operation; 482 was also castrated.

greater than that of the gland first removed. In 9 of the animals an undoubted deficiency of epinephrin developed in the second adrenal. The average of the ratios of the weight of epinephrin contained in the first adrenal to that contained in the second for the 25 animals of the series is 1.27:1. Or, if we add together the weights of epinephrin in the glands first excised, we get 7.86 mgm., as compared with 6.42 mgm. in the glands removed at the end of the experiment, giving a ratio of 1.22:1. The variability of the ratio in the individual experiments (standard deviation, calculated as if the quantities were a number of fortuitously differing quantities, = 0.31) is so great that the probable error of the mean (± 0.042) is greater than in the series of normal rabbits (± 0.031) in spite of the larger number of animals. But in neither series are the ratios well distributed about the mean. Although, therefore, the average depletion in table 2 comes out at about 20 per cent, as compared with 10 per cent in table 1, it would be risky to draw from this the conclusion that trauma has a greater effect in inducing post-operative depletion in the epinephrin store in the thyro-parathyroidectomised rabbits. More weight might perhaps be given to the fact, that the number of animals in which an undoubted depletion was observed was twice as great in these as in the normal rabbits, and also to the fact that the degree of depletion, when it was present, was apt to be greater. It might be thought possible that if the general metabolism of the animal was depressed by the loss of the thyroids, the formation of epinephrin might also be slowed, and that this might be accentuated by the operation while the liberation of epinephrin went on. A depletion of the store would then result, and be more or less marked, of course, in different individuals. It is also not beyond the bounds of possibility that changes in the sympathetic nervous system, associated with the removal of the thyroids and the chief part of the parathyroids, might render it easier for post-operative depletion of the epinephrin store to become evident even in the absence of a general anesthetic.

There is another factor, however, which complicates the matter, apart from the relatively small number of experiments. It will be seen from tables 1 and 2 that while the average bodyweight of the normal rabbits was somewhat greater than that of the thyro-parathyroidectomised rabbits, the average weight of the adrenals was more than 50 per cent greater in the latter group. The normal animals had an average of 176 mgm. of adrenal per kilogram of bodyweight and the thyroidectomised rabbits an average of 287 mgm., a difference

of over 60 per cent. Both averages are somewhat too low, as it had not been intended to use the data for any other purpose than comparison of the epinephrin stores of the two adrenals, and the animals were therefore weighed without removal of the gastro-intestinal contents. Considerable error in calculating the relative weight of an organ in terms of bodyweight is introduced if the intestinal contents are included in the bodyweight, especially in an animal like the rabbit where they vary so greatly in amount. There can be no serious error, however, in comparing the average results in tables 1 and 2, because in both series the bodyweights are the gross weights and the errors due to inclusion of the gastro-intestinal contents would, on the average, be about the same in each. The animals were all fed on the same diet.

Gley (5) gives the average total weight of the adrenals in normal rabbits as 0.25 gram, a much smaller weight than in our animals. The weights of the rabbits, so far as he has given them, are on the average about the same as those in table 1. We are unable to account for this discrepancy, but recognize that the variability of the adrenal weight in animals of the same size is considerable. Herring (6) gives the average as 0.20 gram adrenal per kilogram bodyweight for 3 normal rabbits.

The weight of epinephrin per gram of adrenal in the adrenal first removed, and in which little, if any, depletion due to the operation could have occurred, was on the average 1.01 mgm. in the normal and 0.97 mgm. in the thyro-parathyroidectomised rabbits. Putting it in another way, the total epinephrin in the adrenals removed first in table 1 was 3.17 mgm. and the total weight of the adrenals 3.289 grams, i.e., 0.96 mgm. epinephrin per gram of adrenal. The total epinephrin in the glands first excised in table 2 was 7.86 mgm. and the total weight of these glands 7.718 grams, i.e., 1.02 mgm. epinephrin per gram adrenal. In other words, the hypertrophy of the adrenals in the second group has been accompanied by an almost precisely proportional increase, on the average, of epinephrin. The simplest assumption to explain this would be that the medulla had hypertrophied *pari passu* with the cortex. But this does not necessarily follow. It might be that the medullary cells, without increase in number or total mass, were on the average better filled with epinephrin in the hypertrophied glands, either because of the increased blood flow or for other reasons. The percentage of epinephrin in the largest adrenals may be even somewhat greater than the average. Thus, in table 2 there are 8 rabbits, the first removed adrenal of which weighed more than 0.300 gram.

The total weight of the 8 adrenals was 3.809 grams (average 0.476 gram) and the total weight of epinephrin in them 4.40 mgm. (1.15 mgm. epinephrin per gram adrenal). Apart from the fact that when the epinephrin store is large the error in its estimation is less than when it is small, and that the ratio measuring the depletion can therefore be more exactly estimated, it is conceivable that the large and better filled adrenals may more easily part with a portion of the epinephrin store after operation than the small adrenals with a smaller store.

Be this as it may, the possibility should be considered that it is not the loss of the thyroids and parathyroids as such but the adrenal hypertrophy, however produced, with the associated enhancement of the total epinephrin store, which may be responsible for the apparently greater tendency to post-operative depletion, in the absence of a general anesthetic. For instance, if we compare animals with adrenals within the same range of weight (0.2 to 0.3 gram) from tables 1 and 2, it appears that in 9 normal rabbits the average of the ratios representing the epinephrin in the adrenal first removed to that in the adrenal last removed was 1.16. The average weight of the adrenal first excised was 0.238 gram. In 14 thyroidectomised rabbits with adrenals weighing between 0.2 and 0.3 gram, the corresponding average ratio was 1.20 and the average weight of the adrenal first removed 0.254 gram. Here there was practically no difference between the two groups in the tendency to post-operative depletion. It must be pointed out also that in both tables there are animals with large adrenals and correspondingly large loads of epinephrin which show no depletion whatever.

At first glance it might appear that the tendency to post-operative depletion was greater when the peritoneum had been opened than when the first adrenal was removed by an extraperitoneal operation. If trauma, as such, is really an important factor in the depletion, it might be argued that the depletion should be greater and more frequent after the (supposedly) more severe intraperitoneal operation. It so happens, however, what could not have been known till the animals had been operated on, that the average hypertrophy, or at least the average weight of the adrenal in proportion to the bodyweight, was 40 to 50 per cent greater and the epinephrin load correspondingly greater in the animals (in table 2) subjected to the intraperitoneal operation than in the others. The 12 rabbits operated upon through the peritoneum had a total bodyweight of 26.88 kgm. and a total adrenal weight of 8.988 grams (average 0.33 gram adrenal per kilogram of bodyweight). If the proportion is calculated for each animal separately

and the average then taken it is 0.34 gram adrenal per kilogram bodyweight. The 13 animals (in table 2) operated upon extraperitoneally had a total bodyweight of 26.51 kgm. and an adrenal weight of 6.374 grams (average 0.24 gram per kilogram, and the same if calculated in the other way). If, then, there is a greater tendency for the big adrenal to exhibit post-operative depletion, when no general anesthetic is given, this may be the effective factor rather than the difference in the nature of the operations. As already remarked, with groups of animals so small and showing such a degree of variability in the results, it is not possible to come to any very definite conclusion on this point. The absolute amount of epinephrin in the adrenals in the animals in table 2 is considerably greater than in the normal rabbits (0.61 mgm. per animal, as against 0.42 mgm.). In making this calculation the epinephrin store of the adrenal first removed has been doubled, except in the instances in which the load of the adrenal excised last was not less than that of the first adrenal, in which case the loads of the two adrenals were simply added. This procedure is necessary because when the second adrenal has a distinctly smaller store than the first it must be assumed that its original store was depleted after the operation.

When the total epinephrin is calculated as a fraction of the bodyweight there is, of course, a marked preponderance in favor of the animals in table 2 (average 0.29 mgm. epinephrin, as against 0.18 mgm. per kilogram of bodyweight).

The most definite conclusion we feel able to draw from our results is that the hypertrophy of the adrenals, whether it is to be attributed solely to the effects of removal of the thyroids and parathyroids or not, carried with it a corresponding increase in the epinephrin store, so that on the average the weight of epinephrin per unit of weight of the gland was at least as great in the big as in the small glands, while the weight of epinephrin per unit of bodyweight was much increased in the animals with adrenal hypertrophy.

In the literature one occasionally encounters the statement that "hypertrophied" adrenals have a much diminished content of epinephrin (for instance after action of various toxins). But from the context it is usually apparent that what is called hypertrophy is really edema, a condition which, of course, produces a marked increase in the weight of the adrenal far more rapidly than a true hypertrophy can do, and is associated with great loss or even total disappearance of the epinephrin store (1). As regards the effects upon the adrenals of removal of the thyroids alone or with the parathyroids, there is very little agreement.

Rogowitch (7) saw no change in the adrenals of rabbits after "complete" thyroidectomy. The animals showed no symptoms which he could associate

with the operation in the 2 to 6 months for which they survived. It cannot be known how much of the parathyroids was removed. He did not weigh the glands and made observations on very few animals.

R. G. Hoskins (8) quotes a number of other observers who obtained a negative result in rabbits. He found (9) that the adrenals of the offspring of guinea pigs thyroidectomised before conception were hypertrophied on an average by 20 per cent (expressed as percentages of the bodyweight). In one successful experiment on a bitch the puppies had hypertrophy of the adrenals. Thyroidectomy in new-born guinea pigs had no effect on the adrenals, so far as could be ascertained at the end of 15 days.

Pick and Pineles (10) saw hypertrophy of the adrenals in 2 goats on which thyroidectomy had been performed at the age of 6 to 10 weeks.

Biedl (11) observed a slight hypertrophy of the adrenal cortex in dogs after removal of the thyroids.

Gley (5) found great hypertrophy of the adrenals of rabbits after thyro-parathyroidectomy when they lived a long time. He did not make an assay of the epinephrin, but compared the effect on the blood pressure of dogs of injecting known amounts of the extract of the glands with the effect of similar amounts from the glands of normal animals. He concluded that the epinephrin content of the adrenals was not diminished, unless, after a long time, the animals were suffering from the condition which he speaks of as "myxoedema."

Tatum (22) has described hyperplasia of the adrenal medulla with increase of fatty material in the cortex, as a result of complete removal of the thyroid in rabbits 2 to 3 weeks old. The inferior parathyroids were not removed.

Carlson (12) says that "after complete thyroidectomy (young rabbits) we invariably get a hypertrophy of the suprarenals to two or three times their normal size."

Herring (6) states that complete thyroidectomy (with removal of the parathyroids) has little, if any, permanent effect upon the adrenalin content of the suprarenals of the rabbit, although he points out that his experiments were not numerous enough (5 rabbits allowed to live 30 to 73 days after operation). Also there seemed to be no definite effect upon the adrenal weight. None of the animals showed any symptoms.

It has been stated by various observers that feeding with thyroid substance,—R. G. Hoskins (13), Herring (14), E. R. Hoskins (15), Hewitt (16)—or injecting with an ether-soluble material derived from the thyroid—Iscovesco (17)—causes hypertrophy of the adrenals in various animals (guinea pigs, rats, rabbits, cats). Herring also concluded that the epinephrin content of the adrenals and especially the weight of epinephrin in proportion to the bodyweight are increased. Kuriyama (18) maintains that the variations found by Herring both in the adrenal weight and epinephrin content fall within the normal range, and could find no evidence that in the rat there was any special influence of thyroid feeding. In any case, thyroid substance is stated to cause an increase in size in so many different organs, that hypertrophy of the adrenal, associated with thyroid feeding, would be no indication that any special physiological relationship exists between the thyroid and the adrenals, and would certainly be no indication that hypertrophy of the adrenals might not also be associated with thyroidectomy. Many conditions would seem to affect the size of these glands. Thus Guieyesse (19),

Watrin (20) and other observers consider their hypertrophy during pregnancy a normal physiological event. Hatai (21) has asserted that they hypertrophy in female rats, subjected to prolonged muscular exercise daily for several months.

SUMMARY

The question of the relative importance of the anesthetic and the trauma in causing post-operative depletion of the epinephrin store of the adrenals, was tested by removing one adrenal (in rabbits) under local anesthesia (ethyl chloride) and the other after killing the animal after an interval of 5 to 7 hours. In 12 out of 15 rabbits no evidence of depletion was found. In the remaining three there seems to have been a genuine moderate depletion of the second adrenal as compared with the first. In one cat there was no depletion. The post-operative depletion observed in operations under general anesthesia appears, therefore, to be related to the anesthesia in a greater degree than to the trauma.

In another series of 25 rabbits which were handed over to us by Dr. David Marine, and on which thyro-parathyroidectomy had been performed, similar observations were made on the epinephrin store. The average weight of the adrenals in these animals in proportion to the bodyweight was considerably greater than in the normal rabbits. The average weight of epinephrin per unit weight of adrenal was the same in these animals as in the normal series. The store had therefore increased on the average in the same proportion as the gland weight. The average weight of epinephrin per kilogram of bodyweight was markedly greater than in the normal series.

Sixteen of the 25 rabbits showed no evidence of post-operative depletion. In the remaining 9 animals there was an undoubted deficiency in the epinephrin store of the adrenal last removed, as compared with its fellow.

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